(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/08660 A2

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/US00/20292

(22) International Filing Date: 26 July 2000 (26.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/366,314 2 August 1999 (02.08.1999) US

(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 3003 South State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PHILBERT, Martin, A.** [GB/GB]; 48311 Hill Top Drive East, Plymouth, MI 48170 (US). **TJALKENS, Ronald** [US/US]; 4259 Dexter Road, Ann Arbor, MI 48103 (US). **AYLOTT, Jonathan, W.** [US/US]; 2170 Cram Place #13, Ann Arbor, MI 48105 (US). **CLARK, Heather, A.** [US/US]; 168 Dove Lane, Middletown, CT 06457 (US). **MONSON, Eric, E.** [US/US]; 2025 Huron Parkway #211, Ann Arbor, MI 48104 (US). **KOPELMAN, Raoul** [US/US]; 1065 Heatherway Street, Ann Arbor, MI 48104 (US).

(74) Agents: **CARROLL, Peter, G.** et al.; Medlen & Carroll, LLP, Suite 2200, 220 Montgomery Street, San Francisco, CA 94104 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/08660 A2

(54) Title: TARGETED FIBERLESS RADIATIVE EFFECTORS

(57) Abstract: The present invention is related to cell or pathogen destruction via fiberless radiative effectors that encapsulate a free radical generator. Fiberless radiative effectors include a polymer matrix, a photodynamic or radiodynamic dye which produces free radicals upon stimulation, cloaking material, and at least one molecular recognition element for targeting to a biological target. Other fiberless radiative effectors may be used for imaging biological targets, and include a compound which is detectable by an imaging system, such as luciferase or iron.

TARGETED FIBERLESS RADIATIVE EFFECTORS

FIELD OF THE INVENTION

The present invention is related to cell or pathogen destruction via fiberless radiative effectors that encapsulate a free radical generator.

BACKGROUND OF THE INVENTION

Despite the enormous efforts and resources directed at finding a cure, cancer remains an elusive and deadly foe for mankind. In the United States, more than 900,000 new cases of cancer are diagnosed each year, and more than 600,000 people die from cancer every year. The standard methods of treatment usually include chemotherapy, radiation treatment, and surgical removal of tumors and/or growths, or some combination thereof. These treatments, combined with an emphasis on preventative lifestyle modification, have afforded a measure of success in the battle against some cancers. However, cancer remains one of the leading causes of mortality, and cancers detected at matured stages are typically fatal.

Numerous chemical agents have been devised for the treatment of cancer with varying degrees of efficacy. However, no single drug has one hundred percent effectiveness against different cancers, and negative side-effects ranging from minor to serious are always present.

One class of chemical agents that has found some use against cancer are photosensitizers. Such compounds are used for photodynamic therapy. Photodynamic therapy requires that the photosensitizer accumulate in the tumor. Local activation of the photosensitizer is accomplished by delivery of visible light to the tumor, generally via a laser. The primary mechanism of action is the *in situ* generation of an active form of molecular oxygen (singlet oxygen) that causes vascular stasis followed by vascular hemorrhage and tumor wall destruction. This process appears to be mediated by cytokines including prostaglandins, lymphokines, and thromboxanes. For example, the photosensitizer Photofrin II (porfimer sodium) has been used for the treatment of bladder cancer, endobronchial non-small cell cancer, retroperitoneal sarcoma, and malignant dysphagia in esophogeal cancer (*See, e.g.*, Dougherty and Marcus, Eur. J. Cancer, 28A(10):1734-42 [1992]).

Conventional photodynamic therapy has several significant limitations. Photofrin and other photosensitizers are normally given intravenously and do not specifically target tumors. Malignant and transformed cells do not demonstrate more selective or higher drug uptake than their normal counterparts. Therefore, the light source must be directed to the tumor and not surrounding tissue, which may also be damaged. Packaging Photofrin in liposomes (Jiang *et al.*, Photochem. Photobiol., 67:23S [1998]) does not improve selectivity because the liposomes bind to and deposit Photofrin in normal cells as well. Furthermore, systemic administration of photosensitizers causes cutaneous photosensitivity that can last from six to eight weeks, and may lead to the development of contracted bladder due nonselective accumulation (Xioa *et al.*, Photochem. Photobiol. 67(5):573-83 [1998]). Attempts to solve these problems by utilizing intravesical (*i.e.*, i.b.) administration have had mixed results. Also, because of the nonselectivity of current photosensitizers, relativity large amounts must be administered, resulting in a low therapeutic index. Finally, the use of photodyanamic therapy for some tumors is limited because of the inability to deliver light to the tumors.

Clearly, there is need for alternative strategies and reagents for photodynamic therapy. It would be desirable if such reagents could be administered in low doses, so that collateral damage of adjacent tissues or cells is low. It would also be desirable if the reagents and methods did not cause cutaneous photosensitivity.

SUMMARY OF THE INVENTION

The present invention is related to pathogen and cancer cell destruction via fiberless radiative effectors that encapsulate a free radical generator or toxic agent release system. Such effectors find use *in vivo* and *in vitro*.

In some embodiments, the present invention provides a fiberless radiative effector that has molecular recognition elements fixed thereto. In certain embodiments of the present invention, the fiberless radiative effector is toxic to or includes a system for generating molecules toxic to living cells.

It is not intended that the present invention be limited by the precise composition of the fiberless radiative effectors. Indeed, a variety of compositions are contemplated. The

fiberless radiative effectors of the present invention are preferably either solid or semisolid particles (*e.g.*, nanospheres) preferably ranging in size between approximately 10 micrometers and 0.1 nanometers, and more preferably, between approximately 5 micrometers and 1 nanometer in diameter. In some embodiments of the present invention, the ultimate size of the fiberless radiative effectors is attained by fine grinding and filtering or by micro/nano-emulsion techniques used to form mono-disperse colloidal particles (*i.e*, rather than nano-fabrication). In some embodiments, the fiberless radiative effector is selected from polymer fiberless radiative effectors acrylamide fiberless radiative effectors, and sol-gel fiberless radiative effectors.

It is not intended that the fiberless radiative effectors of the present invention be limited to a particular polymer. Indeed, a variety of polymers are contemplated. In some embodiments of the present invention, the polymer is selected from poly(vinyl chloride), poly(vinyl chloride) carboxylated, and poly(vinyl chloride-co-vinyl acetate-co-vinyl alcohol). In some preferred embodiments, the fiberless radiative effectors further comprise an additive and a plasticizer. In other embodiments, the present invention provides acrylamide radiative fiberless effectors comprising N,N-methylene-bi(acrylamide) polymerized into a gel.

It is not intended that the present invention be limited by the nature of the molecular recognition element. Indeed, a variety of molecular recognition elements are contemplated. In some embodiments, the molecular recognition element is a carbohydrate or a carbohydrate-binding protein (*e.g.*, a lectin). In some embodiments of the present invention, the molecular recognition element is a polypeptide. In some embodiments of the present invention, the polypeptide is preferably an antigen binding protein. In further embodiments of the present invention, the antigen binding protein is selected from antibodies, humanized antibodies, F(ab) fragments, $F(ab)_2$ fragments, and single chain antibodies. In other embodiments of the present invention, the polypeptide is preferably an agonist or antagonist that binds to a cell surface receptor. In still other embodiments of the present invention, the polypeptide is preferably avidin. In still other embodiments of the present invention, the polypeptide is preferably a signal sequence that directs the fiberless radiative effector to an intracellular compartment (*e.g.*, mitochondria or nucleus). In some embodiments of the present invention, the molecular recognition elements comprises a nucleic acid. In some preferred embodiments, the nucleic

acid is selected from RNA and DNA. In still further embodiments of the present invention, the molecular recognition element is preferably an organic molecule. In some embodiments, the organic molecule is preferably biotin.

It is not intended that the fiberless radiative effectors of the present invention be limited to any particular system for generating toxic molecules (*e.g.*, singlet oxygen, hydroxyl radicals, and superoxide radicals or nitric oxide) or level of generated toxicity. Indeed a variety of toxicities and systems for generating toxic molecules are contemplated. In some embodiments, the fiberless radiative effector further comprises a system for generating toxic free radicals, preferably oxygen or nitrogen free radicals. In further embodiments, the system for generating free radicals comprises a photodynamic dye. It is not intended that the present invention be limited to any particular photodynamic compound. Indeed, a variety of photodynamic compounds are contemplated. In some embodiments, the photodynamic compound are selected from Photofrin, ruthenium red compounds (*i.e.*, Ru-diphenyl-phenanthroline and Tris(1-10-phenanthroline)ruthenium(II) chloride), tin ethyl etiopurpurin, protoporphyrin IX, chloroaluminum phthalocyanine, tetra(M-hydroxyphenyl)chlorin.

In other embodiments of the present invention, the system for generating free radicals comprises a radiodynamic (*i.e.*, scintillating) compound. It is not intended that the present invention be limited to any particular radiodynamic compound. Indeed, a variety of radiodynamic compounds are contemplated. In some embodiments, the radiodynamic compounds are selected from NaI-125, 2,5-diphenyloxazole (PPO); 2-(4-biphenyl)-6-phenylbenzoxazole; 2,5-bis-(5'-tert-butylbenzoxazoyl-[2'])thiophene; 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole; 1,6-diphenyl-1,3,5-hexatriene; trans-p,p'-diphenylstilbene; 2-(1-naphthyl)-5-phenyloxazole; 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole; p-terphenyl; and 1,1,4,4-tetraphenyl-1,3-butadiene. In some embodiments, the radiodynamic system for generating toxic materials further comprises a supra- or super-molecular antenna system (*e.g.*, styrene, polystyrene, or poly(styrene)-poly(vinylpyridine). In other embodiments, the radiodynamic system for generating toxic materials further comprises a photodynamic compound (*e.g.*, porphyrin, Photofrin, hematoporphyrin).

In some embodiments of the present invention, the fiberless radiative effector further comprises a radioactive element. The present invention is not limited to the nature of the radioactive element. Indeed, a variety of radioactive elements are contemplated. In some embodiments, the radioactive elements are selected from elements or compounds that emit gamma rays (*e.g.*,^{111}In-oxine, ^{59}Fe, ^{67}Cu, ^{125}I, and ^{51}Cr) or compounds that emit beta particles (*e.g.*, ^{32}P, ^{3}H, ^{35}S, ^{14}C).

In some embodiments, the fiberless radiative effector further comprises a cloaking agent or means. The present invention is not limited to any particular cloaking agent or means. Indeed, a variety of cloaking agents or means are contemplated. In some embodiments, the cloaking agent or means is a liposome in which the fiberless effectors are packaged. In other embodiments, the cloaking agent or means is a red blood cell in which the fiberless effectors are packaged. In still further embodiments, the cloaking agent or means is preferably a cloaking film. A variety of cloaking agents are contemplated, including, but not limited to the following: poly(ethylene glycol), poly(acrylamide), poly(vinyl pyrrolidone), poly(ethylene glycol)-phosphatidyl.

The fiberless radiative effectors of the present invention are utilized to destroy target organisms, cells or proteins or other macromolecules. The present invention is not limited to the destruction of any particular target organism. Indeed, a variety of target organisms are contemplated. In some embodiments, the target organism is preferably a prion or bacterial organism (*e.g.*, *Legionella peomophilia*, *Mycobacterium tuberculosis*, *Clostridium tetani*, *Hemophilus influenzae*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Bacillus anthracis*, *Vibrio cholerae*, *Borrelia burgdorferi*, *Cornebacterium diphtheria*, *Staphylococcus aureus*, and the like). In other embodiments, the target organism is preferably a virus (*e.g.*, human papilloma virus, human immunodeficiency virus, rubella virus, polio virus, and the like). The present invention is also not limited to any particular target cell. Indeed, a variety of target cells are contemplated. In some embodiments, the target cells are cancer cells selected from topical cells (*e.g*, malignant melanoma cells and basal cell carcinomas), ductal cells (*e.g.*, mammary ductal adenocarcinoma cell and bowel cancer cells), and deep tissue cells (*e.g.*, hepatocellular carcinoma cells, CNS primary lymphoma cells, and glioma cells). In some

embodiments, the target cell or organism includes a target epitope. The target epitopes of the present invention are not limited to any particular epitopes. Indeed, a variety of epitopes are contemplated. In some embodiments, the target epitope is selected from cell surface proteins, cell surface receptors, cell surface polysaccharides, extracellular matrix proteins, intracellular proteins and intracellular nucleic acids. In still other embodiments, the effector is targeted via a signal peptide to a particular cellular organelle (*e.g.*, mitochondria or the nucleus).

Accordingly, the present invention provides a method for destroying target cells, macromolecules or organisms. In some embodiments of the present invention, the method comprises providing a target cell or organism and a first fiberless radiative effector comprising a molecular recognition element. In further embodiments of the present invention, the target cell or organism and the fiberless radiative effector are combined so that the molecular recognition element binds to the target cell or organism to form a first effector-target complex. The present invention is not limited by the nature of complex formation. Indeed a variety of complexes are contemplated. In some embodiments, the fiberless radiative effector binds to a cell surface protein, a cell surface receptor, a extracellular matrix protein, a viral coat protein, a bacterial cell wall protein, a viral or bacterial polysaccharide, or be directed to a particular organelle within a cell. In some embodiments of the present invention, the fiberless radiative effector further comprises a photodynamic dye. In still further embodiments, the first effector-target complex is exposed to light so that singlet oxygen is produced. In particularly preferred embodiments, the singlet oxygen causes oxidation and necrosis/apoptosis of the target cell, macromolecule, or organism.

In another embodiment, the present invention provides a method for destroying a biological target via a stacking approach. In some embodiments of the present invention, the method comprises providing a first fiberless radiative effector comprising a first molecular recognition element and a stacking epitope, a second fiberless radiative effector comprising a second molecular recognition element, and a biological target comprising a target epitope. In further embodiments of the present invention, one of the first and second fiberless radiative effectors further comprises a radiodynamic or photodynamic dye, while the other of the first and second radiative fiberless effectors comprises a radioactive element that emits energy (*e.g.*, alpha particles, beta particles, or gamma rays). In some embodiments, the release of

alpha or beta particles or gamma rays by the radioactive element causes the excitation of the radiodynamic or photodynamic dye, causing the production of singlet oxygen or oxygen free radicals. In some embodiments of the present invention, the biological target, first fiberless radiative effector and second fiberless radiative effector are combined so that the first fiberless radiative effector binds to the target epitope on the biological target and the second fiberless radiative effector binds to the stacking epitope on the first fiberless radiative effector. The binding of the first fiberless radiative effector to the second radiative fiberless effector brings the radioactive element into close proximity with the radiodynamic or photodynamic dye, resulting in the production of singlet oxygen which causes the oxidation of macromolecules and necrosis/apoptosis of the target cell.

In still another embodiment, the present invention provides an alternative stacking approach. This approach allows a universal fiberless radiative effector (*e.g.*, a fiberless radiative effector with either avidin or biotin as its molecular recognition element) to be used to destroy a variety of biological targets. In some embodiments of the present invention, the method comprises providing a first fiberless radiative effector comprising a first molecular recognition element (*e.g.*, avidin), a biological target comprising a target epitope, and a stacking reagent (*e.g.*, a biotinylated antigen binding protein) comprising a molecular recognition element and at least one stacking epitope. In some embodiments of the present invention, the first fiberless radiative effector comprises a photodynamic or radiodynamic dye. In other embodiments, a second fiberless radiative effector is provided that comprises a radioactive element that emits energy. In some embodiments of the present invention, the target cell, stacking reagent and first fiberless radiative effector are combined so that the stacking reagent molecular recognition element binds to the biological target and the fiberless radiative effector molecular recognition element binds to the stacking reagent epitope to form a target cell-stacking reagent-fiberless radiative effector complex. In some embodiments, the complex is exposed to a light source that excites the photodynamic dye so that singlet oxygen is produced which causes oxidation and necrosis of the target cell. In other embodiments, a second fiberless radiative effector comprising a radioactive element and a second molecular recognition element is provided. The second molecular recognition element preferably binds to a second stacking epitope on the stacking reagent so that a complex is formed. In some

preferred embodiments, the alpha or beta particles emitted by the radioactive element in the second fiberless radiative effector excite the radiodynamic or photodynamic dye in the first fiberless radiative effector so that singlet oxygen is produced.

In other embodiments of the present invention, methods for detecting cells or organisms are provided. In some embodiments, a fiberless radiative effector comprising a fluorescent photosensitizer or dye and a molecular recognition element and a biological target are provided. In some embodiments, the fiberless radiative effector and biological target are combined so that the molecular recognition element binds to the biological target to form a target-fiberless radiative effector complex. In other embodiments, the presence of the fiberless radiative effector is detected by exposing the complex to light of the appropriate wavelength that causes the photosensitizer or dye to fluoresce. In still further embodiments, fluorescence is detected by a luminometer, by a microscope equipped for epifluorescence, or by capturing a digital image from a microscope with a CCD camera. In still further embodiments, the fluorescent signal is quantitated by determining pixel intensity.

DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic composite (photodynamic/radiodynamic) depiction of a fiberless radiative effector.

Fig. 2 is a photomicrograph of cells that were lipofected with fiberless radiative effectors.

Fig. 3 is a photomicrograph demonstrating lipofection with PEBBLEs.

Fig. 4 depicts plots of tumor volumes obtained form serial MRI versus time following 9L cell implantation.

Fig. 5 lists the sequences for (A) wild-type IL13 cDNA, (B) wild-type IL13 protein, and the protein sequences for the Il13 variants (C) hIL13.E13K and (D) hIL13.E13Y.

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

As used herein, the term "fiberless radiative effector" refers to a polymeric particle (*i.e.*, a particle as opposed to a fiber) or particle comprising a porous inorganic glass (*e.g.*,

sol-gel that is light penetrable (*i.e.*, by photons) or penetrable to some other energy source (*e.g.* or β particles or gamma rays) and contains a toxic agent. The terms "core" and "core matrix" when used in reference to fiberless radiative effectors refer to the polymeric or porous metal oxide portion of the fiberless radiative effector.

As used herein, the term "nanosphere" refers to a polymeric or sol-gel particle of about approximately 0.5 nanometers to 10 micrometers in diameter.

As used herein, the term "molecular recognition element" refers to molecular capable of binding to, hybridizing to, or otherwise interacting with another molecule. Examples of molecular recognition elements include, but are not limited to, nucleic acid molecules (*e.g.*, RNA and DNA, including ligand-binding RNA molecules), polypeptides (*e.g.*, antigen binding proteins, receptor ligands, signal peptides, hydrophobic membrane spanning domains), and organic molecules (*e.g.*, biotin). A given fiberless radiative effector may have affixed thereto one or a variety of molecular recognition elements.

As used herein, the term "toxic agent" refers to a material or mixture of materials which are themselves toxic to a biological system (*e.g.*, pathogen, virus, bacteria, cell, or multicellular organism) or which upon a stimulus (*e.g.*, light, α or β particles) produce an agent (*e.g.*, singlet oxygen or free radical) which is toxic to a biological system.

As used herein, the terms "cloaking material" and "cloaking agent" refers to any material used to protect a fiberless radiative effector from recognition by the reticulo-endothelial system, including, but not limited to poly(ethylene glycol) and liposomes.

As used herein, the term "stacking epitope" refers to a material (*e.g.*, biotin) on a first fiberless radiative effector that is recognized by the molecular recognition element of a second fiberless radiative effector or some other material (e.g., avidin or antigen binding protein).

As used herein, the term "stacking reagent" refers to a material that binds to a biological target and is recognizable by the molecular recognition element of a fiberless radiative effector.

As used herein, the term "stacking reagent epitope" refers to a material (*e.g.*, biotin or avidin) on a stacking reagent that is recognizable be a molecular recognition element.

As used herein, the term "antigen binding protein" refers to proteins which bind a specific antigen. "Antigen binding proteins" include, but are not limited to, immunoglobulin, polyclonal, monoclonal, chimeric, single chain, and humanized antibodies, Fab fragments, F(ab')2 fragments, and Fab expression libraries.

As used herein the term "biological target" refers to any organism, cell, microorganism, bacteria, virus, fungus, plant, prion, protozoa, or pathogen or portion of an organsim, cell, microorganism, bacteria, virus, fungus, plant, prion, protozoa or pathogen.

As used herein, the terms "peptide" or "polypeptide" refer to a chain of amino acids (*i.e.*, two or more amino acids) linked through peptide bonds between the α-carboxyl carbon of one amino acid residue and the α-nitrogen of the next. A "peptide" or "polypeptide" may comprise an entire protein or a portion of protein. "Peptides" and "polypeptides" may be produced by a variety of methods including, but not limited to chemical synthesis, translation from a messenger RNA, expression in a host cell, expression in a cell free translation system, and digestion of another polypeptide.

As used herein the term "protein" is used in its broadest sense to refer to all molecules or molecular assemblies containing two or more amino acids. Such molecules include, but are not limited to, proteins, peptides, enzymes, antibodies, receptors, lipoproteins, and glycoproteins.

As used herein, the term "enzyme" refers to molecules or molecule aggregates that are responsible for catalyzing chemical and biological reactions. Such molecules are typically proteins, but can also comprise short peptides, RNAs, ribozymes, antibodies, and other molecules.

As used herein, the terms "nucleic acid" or "nucleic acid molecules" refer to any nucleic acid containing molecule including, but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-

methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

Nucleic acid molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotides or polynucleotide, referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between

nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (*i.e.*, identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target that lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.).

When used in reference to a double-stranded nucleic acid sequence such as a cDNA to an RNA of the present invention, the term "substantially homologous" refers to any probe that

can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids.

As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*See e.g.*, Anderson and Young, Quantitative Filter Hybridization, *in Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m.

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Those skilled in the art will recognize that "stringency" conditions may be altered by varying the parameters just described either individually or in concert. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences (*e.g.*, hybridization under "high stringency" conditions may occur between homologs with about 85-100% identity, preferably about 70-100% identity). With medium stringency conditions, nucleic acid base pairing will occur between nucleic acids with an

intermediate frequency of complementary base sequences (*e.g.*, hybridization under "medium stringency" conditions may occur between homologs with about 50-70% identity). Thus, conditions of "weak" or "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

As used herein, the term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 100 residues long (*e.g.*, between 15 and 50), however, as used herein, the term is also intended to encompass longer polynucleotide chains. Oligonucleotides are often referred to by their length. For example a 24 residue oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

As used herein, the term "polymer" refers to material comprised of repeating subunits. Examples of polymers include, but are not limited to polyacrylamide and poly(vinyl chloride), poly(vinyl chloride) carboxylated, and poly(vinyl chloride-co-vinyl acetate co-vinyl) alcohols.

As used herein, the term "polymerization" encompasses any process that results in the conversion of small molecular monomers into larger molecules consisting of repeated units. Typically, polymerization involves chemical crosslinking of monomers to one another.

As used herein, the term "plasticizer" refers to organic compounds added to a polymer to modify it by internal modification (*i.e.*, solvation). The plasticizer replaces some secondary valence bonds between polymer subunits with plasticizer-to-polymer bonds. Examples of plasticizers include, but are not limited to, benzyl ether, benzyl 2-nitrophenyl ether, 1-decanol, 2-nitrodiphenyl ether, 1-octodecanol, and the like.

As used herein, the terms "photosensitizer," and "photodynamic dye," refer to materials which undergo transformation to an excited state upon exposure to a light quantum (hv). Examples of photosensitizers and photodynamic dyes include, but are not limited to, Photofrin 2, benzoporphyrin, m-tetrahydroxyphenylchlorin, tin etiopurpurin, copper benzochlorin, and other porphyrins.

As used herein, the term "radiodynamic compound" refers to materials which undergo a transition an excited state upon exposure to x-rays, gamma ray, or beta particles. Examples of radiodynamic compounds include, but are not limited to polystyrene and scintillating compounds (*e.g.*, NaI-125, 2,5-diphenyloxazole (PPO); 2-(4-biphenyl)-6-phenylbenzoxazole; 2,5-bis-(5'-tert-butylbenzoxazoyl-[2'])thiophene; 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole; 1,6-diphenyl-1,3,5-hexatriene; trans-p,p'-diphenylstilbene; 2-(1-naphthyl)-5-phenyloxazole; 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole; p-terphenyl; and 1,1,4,4-tetraphenyl-1,3-butadiene)

As used herein, the term "radioactive element" refers to any material which emits α, β, or γ particles.

As used herein, the term "biological target" refers to any cell, bacterium, virus, or portion thereof, including, but not limited to, cell walls, cell surface receptors, extracellular matrix proteins, viral coat proteins, nucleic acids *(e.g.*, RNA and DNA), proteins, and organelles (*e.g*, mitochondria and nuclei).

As used herein, the term "target-fiberless radiative effector complex" or simple "target-effector complex" refers to at least one fiberless radiative effector bound to, contained in, or otherwise affixed to a biological target.

As used herein, the term "reaction" refers to any change or transformation in which a substance (*e.g.*, molecules, membranes, and molecular assemblies) combines with other substances, interchanges constituents with other substances, decomposes, rearranges, or is otherwise chemically altered. As used herein, the term "reaction means" refers to any means of initiating and/or catalyzing a reaction. Such reaction means include, but are not limited to, enzymes, temperature changes, and pH changes. The phrase "affinity for said reaction means" refers to compounds with the ability to specifically associate (*e.g.*, bind) to a given reaction mean, although not necessarily a substrate for the reaction means. For example, a PLA_2 antibody has affinity for PLA_2, but is not the substrate for the enzyme.

As used herein, the term "immobilization" refers to the attachment or entrapment, either chemically or otherwise, of material to another entity (*e.g.*, a solid support) in a manner that restricts the movement of the material.

As used herein, the terms "material" and "materials" refer to, in their broadest sense, any composition of matter.

As used herein, the term "antigen" refers to any molecule or molecular group that is recognized by at least one antibody. By definition, an antigen must contain at least one epitope (*i.e.*, the specific biochemical unit capable of being recognized by the antibody). The term "immunogen" refers to any molecule, compound, or aggregate that induces the production of antibodies. By definition, an immunogen must contain at least one epitope.

As used herein the term "antibody" refers to a glycoprotein evoked in an animal by an immunogen (antigen). An antibody demonstrates specificity to the immunogen, or, more specifically, to one or more epitopes contained in the immunogen. Native antibody comprises at least two light polypeptide chains and at least two heavy polypeptide chains. Each of the heavy and light polypeptide chains contains at the amino terminal portion of the polypeptide chain a variable region (*i.e.*, VH and VL respectively), which contains a binding domain that interacts with antigen. Each of the heavy and light polypeptide chains also comprises a constant region of the polypeptide chains (generally the carboxy terminal portion) which may mediate the binding of the immunoglobulin to host tissues or factors influencing various cells of the immune system, some phagocytic cells and the first component (C1q) of the classical complement system. The constant region of the light chains is referred to as the "CL region," and the constant region of the heavy chain is referred to as the "CH region." The constant region of the heavy chain comprises a CH1 region, a CH2 region, and a CH3 region. A portion of the heavy chain between the CH1 and CH2 regions is referred to as the hinge region (*i.e.*, the "H region"). The constant region of the heavy chain of the cell surface form of an antibody further comprises a spacer-transmembranal region (M1) and a cytoplasmic region (M2) of the membrane carboxy terminus. The secreted form of an antibody generally lacks the M1 and M2 regions.

As used herein, the term "sol-gel" refers to preparations composed of porous metal oxide glass structures. Such structures can have biological or other material entrapped within the porous structures. The phrase "sol-gel matrices" refers to the structures comprising the porous metal oxide glass with or without entrapped material. The term "sol-gel material" refers to any material prepared by the sol-gel process including the glass material itself and

any entrapped material within the porous structure of the glass. As used herein, the term "sol-gel method" refers to any method that results in the production of porous metal oxide glass. In some embodiments, "sol-gel method" refers to such methods conducted under mild temperature conditions. The terms "sol-gel glass" and "metal oxide glass" refer to glass material prepared by the sol-gel method and include inorganic material or mixed organic/inorganic material. The materials used to produce the glass can include, but are not limited to, aluminates, aluminosilicates, titanates, ormosils (organically modified silanes), and other metal oxides.

As used herein, the term "selective binding" refers to the binding of one material to another in a manner dependent upon the presence of a particular molecular structure (*i.e.*, specific binding). For example, a receptor will selectively bind ligands that contain the chemical structures complementary to the ligand binding site(s). This is in contrast to "non-selective binding" or "background binding," whereby interactions are arbitrary and not based on structural compatibilities of the molecules.

As used herein, the term "pathogen" refers to disease causing organisms, microorganisms, or agents including, but not limited to, viruses, bacteria, parasites (including, but not limited to, organisms within the phyla Protozoa, Platyhelminthes, Aschelminithes, Acanthocephala, and Arthropoda), fungi, and prions.

As used herein, the term "bacteria" and "bacterium" refer to all prokaryotic organisms, including those within all of the phyla in the Kingdom Procaryotae. It is intended that the term encompass all microorganisms considered to be bacteria including *Mycoplasma, Chlamydia, Actinomyces, Streptomyces,* and *Rickettsia.* All forms of bacteria are included within this definition including cocci, bacilli, spirochetes, spheroplasts, protoplasts, *etc.* "Gram negative" and "gram positive" refer to staining patterns obtained with the Gram-staining process which is well known in the art (*See e.g.*, Finegold and Martin, Diagnostic Microbiology, 6th Ed. (1982), CV Mosby St. Louis, pp 13-15).

As used herein, the term "macromolecule" refers to any large molecule such as proteins, polysaccharides, nucleic acids, and multiple subunit proteins. Examples of

macromolecules include, but are not limited to verotoxin I, verotoxin II, Shiga-toxin, botulinum toxin, snake venoms, insect venoms, alpha-bungarotoxin, and tetrodotoxin).

As used herein, the term "virus" refers to infectious agents, which with certain exceptions, are not observable by light microscopy, lack independent metabolism, and are able to replicate only within a host cell. The individual particles (*i.e.*, virions) consist of nucleic acid and a protein shell or coat; some virions also have a lipid containing membrane. The term "virus" encompasses all types of viruses, including animal, plant, phage, and other viruses.

As used herein, the term "membrane receptors" refers to constituents of membranes that are capable of interacting with other molecules or materials. Such constituents can include, but are not limited to, proteins, lipids, carbohydrates, and combinations thereof.

As used herein, the term "drug" refers to a substance or substances that are used to diagnose, treat, or prevent diseases or conditions. Drugs act by altering the physiology of a living organism, tissue, cell, or *in vitro* system that they are exposed to. It is intended that the term encompass antimicrobials, including, but not limited to, antibacterial, antifungal, and antiviral compounds. It is also intended that the term encompass antibiotics, including naturally occurring, synthetic, and compounds produced by recombinant DNA technology.

As used herein, the term "carbohydrate" refers to a class of molecules including, but not limited to, sugars, starches, cellulose, chitin, glycogen, and similar structures. Carbohydrates can also exist as components of glycolipids and glycoproteins.

As used herein, the term "chelating compound" refers to any compound composed of or containing coordinate links that complete a closed ring structure. The compounds can combine with metal ions, attached by coordinate bonds to at least two of the nonmetal ions.

As used herein, the term "molecular recognition complex" refers to any molecule, molecular group, or molecular complex that is capable of recognizing (*i.e.*, specifically interacting with) a molecule. For example, the ligand binding site of a receptor would be considered a molecular recognition complex.

As used herein, the term "room temperature" refers, technically, to temperatures approximately between 20 and 25 degrees centigrade. However, as used generally, it refers to the any ambient temperature within a general area in which an experiment is taking place.

As used herein, the term "encapsulate" refers to the process of encompassing, encasing, or otherwise associating two or more materials such that the encapsulated material is contained and/or immobilized within or onto the encapsulating material.

As used herein, the term "optical transparency" refers to the property of matter whereby the matter is capable of transmitting light such that the light can be observed by visual light detectors (*e.g.*, CCD, photomultipliers, or other detection equipment).

As used herein, the term "organic solvents" refers to any organic molecules capable of dissolving another substance. Examples include, but are not limited to, chloroform, alcohols, phenols, and ethers.

As used herein, term "nanostructures" refers to microscopic structures, typically measured on a nanometer scale. Such structures include various three-dimensional assemblies, including, but not limited to, liposomes, films, multilayers, braided, lamellar, helical, tubular, and fiber-like shapes, and combinations thereof. Such structures can, in some embodiments, exist as solvated polymers in aggregate forms such as rods and coils. The term "solvated polymers" refers to polymer nanostructures that are dissolved in a solvent.

As used herein, the term "liposome" refers to artificially produced spherical lipid complexes that can be induced to segregate out of aqueous media. The terms "liposome" and "vesicle" are used interchangeably herein.

As used herein, the term "biopolymeric liposomes" refers to liposomes that are composed entirely, or in part, of biopolymeric material.

As used herein, the term "ligands" refers to any ion, molecule, molecular group, or other substance that binds to another entity to form a larger complex. Examples of ligands include, but are not limited to, peptides, carbohydrates, nucleic acids (*e.g.*, DNA and RNA), antibodies, or any molecules that bind to receptors.

As used herein, the term "polymeric matrix" refers to matrices whereby some or all of the molecular constituents of the matrix are polymerized.

As used herein, the terms "head group" and "head group functionality" refer to the molecular groups present an the ends of molecules (*e.g.*, the primary amine group at the end of peptides).

As used herein, the term "hydrophilic head-group" refers to ends of molecules that are substantially attracted to water by chemical interactions including, but not limited to, hydrogen-bonding, van der Waals' forces, ionic interactions, or covalent bonds. As used herein, the term "hydrophobic head-group" refers to ends of molecules that self-associate with other hydrophobic entities, resulting in repulsion from water.

As used herein, the term "carboxylic acid head groups" refers to organic compounds containing one or more carboxyl (-COOH) groups located at, or near, the end of a molecule. The term carboxylic acid includes carboxyl groups that are either free or exist as salts or esters.

As used herein, the term "linker" or "spacer molecule" refers to material that links one entity to another. In one sense, a molecule or molecular group can be a linker that is covalently attached two or more other molecules (*e.g.*, linking a ligand to a self-assembling monomer).

As used herein, the term "bond" refers to the linkage between atoms in molecules and between ions and molecules in crystals. The term "single bond" refers to a bond with two electrons occupying the bonding orbital. Single bonds between atoms in molecular notations are represented by a single line drawn between two atoms (*e.g.*, C_8-C_9). The term "double bond" refers to a bond that shares two electron pairs. Double bonds are stronger than single bonds and are more reactive. The term "triple bond" refers to the sharing of three electron pairs. As used herein, the term "ene-yne" refers to alternating double and triple bonds. As used herein the terms "amine bond," "thiol bond," and "aldehyde bond" refer to any bond formed between an amine group (*i.e.*, a chemical group derived from ammonia by replacement of one or more of its hydrogen atoms by hydrocarbon groups), a thiol group (*i.e.*, sulfur analogs of alcohols), and an aldehyde group (*i.e.*, the chemical group -CHO joined directly onto another carbon atom), respectively, and another atom or molecule.

As used herein, the term "covalent bond" refers to the linkage of two atoms by the sharing of at least one electron, contributed by each of the atoms.

As used the term "absorption" refers, in one sense, to the absorption of light. Light is absorbed if it is not reflected from or transmitted through a sample. Samples that appear

colored have selectively absorbed all wavelengths of white light except for those corresponding to the visible colors that are seen.

As used herein, the term "spectrum" refers to the distribution of electromagnetic energies arranged in order of wavelength.

As used herein, the term "magnetocatalysis" refers to cellular apoptosis resulting from exposure to a magnetic field.

As used the term "visible spectrum" refers to light radiation that contains wavelengths from approximately 360 nm to approximately 800 nm.

As used herein, the term "ultraviolet irradiation" refers to exposure to radiation with wavelengths less than that of visible light (*i.e.*, less than approximately 360 nM) but greater than that of X-rays (*i.e.*, greater than approximately 0.1 nM). Ultraviolet radiation possesses greater energy than visible light and is therefore, more effective at inducing photochemical reactions.

As used herein, the term "infrared radiation" refers to exposure to radiation with wavelengths of greater 800 nM.

As used herein, the term "biological organisms" refers to any carbon-based life forms.

As used herein, the terms "signalling material" and "marker" refers to any material or molecule (*e.g.*, luciferase, magnetic particle, or rhodamine) that is detectable by any detection system, including, but not limited to, enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, nuclear magnetic resonance, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or marker.

As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, including biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. These examples are not to be construed as limiting the sample types applicable to the present invention.

As used herein, the term "homobifunctional," refers to a linker molecule with two functional groups that both react with the same chemical group (*e.g.*, primary amines, esters or aledehydes).

As used herein, the term "homobifunctional," refers to a linker molecule with two functional groups that react with different chemical groups (*e.g.*, primary amines, esters or aledehydes).

DESCRIPTION OF THE INVENTION

The present invention is related to cell or pathogen destruction via fiberless radiative effectors that encapsulate a toxic agent or free radical generator.

The fiberless radiative or optical effectors of the present invention comprise one or more toxic agents embedded in a matrix material and a molecular recognition element for targeting the effector to a biological target. The matrix material takes many forms, including, but not limited to polymeric matrices (*e.g.*, poly(acrylamide), poly(vinyl chloride), decyl-methacrylate or poly(lactic acid)) or sol-gel matrices. In preferred embodiments, the fiberless radiative effector is generally spherical, however, the effector takes many shapes and sizes (*e.g.*, helical, tubular, square, or rectangular). In some preferred embodiments, the fiberless radiative effector is about 40 to 400 nm in diameter. A variety of toxic agents are incorporated into the fiberless radiative effector matrix. In preferred embodiments, the toxic agents participate in the production of toxic diffusable molecules (*e.g.*, singlet oxygen, hydroxyl radicals, or superoxide) upon stimulation or excitation from an energy source (*e.g.*, incandescent light, red light, laser, x-rays, or gamma-rays).

A variety of molecular recognition elements are attached to the fiberless radiative effector or used to target the fiberless radiative effector to a biological target. Molecular recognition elements include, but are not limited to, antigen binding proteins, ligands for cell surface receptors, and nucleic acids. The molecular recognition elements are either attached, covalently or otherwise, to the fiberless radiative effector matrix, or it is attached to a cloaking agent that surrounds or coats the fiberless radiative effector. A variety of cloaking agents are utilized with the fiberless radiative effectors of the present invention, including, but

not limited to poly(ethylene glycol), liposomes, poly(ethylene glycol) coated liposomes, and red blood cell ghosts.

The fiberless radiative effectors of the present invention have a number of uses. In some embodiments of the present invention, the fiberless radiative effectors are used to destroy or inhibit the growth of tumor cells. In some embodiments, the RGD peptide is used to target the fiberless radiative effector to the vasculature of head and neck tumors. In other embodiments, IL13 or one of its variants is used to target the fiberless radiative effector to malignant glioma cells. Once the fiberless radiative effectors have bound the tumor cell, the toxic agent acts on the cell. In some embodiments, fiberless radiative effectors containing photodynamic compounds are illuminated, resulting in the production of singlet oxygen and free radicals that diffuse out of the fiberless radiative effector to act on the biological target. In other embodiments, fiberless radiative effectors are used in concert. For example, a first biotinylated fiberless radiative effector containing a radiodynamic compound and a photodynamic compound is attached to a biological target via a molecular recognition element. A second fiberless radiative effector that is conjugated to avidin and contains a gamma-ray emitter is then attached to the first fiberless radiative effector via avidin-biotin binding. The gamma-rays emitted by the second fiberless radiative effector cause production of singlet oxygen and free radicals by the radiodynamic and photodynamic compounds in the first fiberless radiative effector. These toxic molecules then act to destroy or inhibit the growth of the biological target. Fiberless radiative effectors targeted to pathogens are used to destroy the pathogens in a similar manner.

The fiberless radiative effector is also be utilized for imaging tumors or other biological targets. In some embodiments, the fiberless radiative effectors contain a detectable material (*i.e.*, luciferase or magnetic particles) that can be detected by a suitable detection system (*e.g.*, bioluminescent imaging with a CCD or Magnetic Resonance Imaging). In some embodiments the magnetic fiberless radiative effectors also contain a toxic agent, so that the same fiberless radiative effector is used for therapy as well as monitoring the growth of the tumor.

It is not intended that the present invention be limited to a particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the

present invention. However, it is contemplated that the use of the fiberless radiative effectors of the present invention in photodynamic tumor therapy regimes has many advantages over traditional photodynamic therapy. First, there are two critical mass aspects. The large concentration of photoactive centers in the core of the fiberless radiative effector creates a concerted photodynamic effect that destroys the cell (*e.g.*, by "punching" large holes in the membrane to which it is attached or by denaturing receptors/transporters and recognition adhesion molecules). In contrast, the isolated photodynamic molecule has to first penetrate the cell membrane and then enter a certain organelle before it can cause significant damage. Furthermore, as in chemotherapy, isolated photoactive molecules tend to leak out of the cell, be sequestered in the wrong locations, or be pumped back out of cells that express multiple drug resistance proteins. This problem is obviated by the fiberless radiative effector.

In addition, fiberless radiative effectors have several other advantages for tumor therapy. First, no aggregation of the photodynamic molecules occurs so there is no self-quenching of the excitation and no loss in singlet oxygen formation. Second, adding molecular targeting to a photodynamic molecule is quite expensive and often impractical due to interference between the two main functions - targeting and photodynamic activity. The fiberless radiative effectors of the present invention circumvent these problems and deliver many more killer radicals per target. Third, the side effects from photodynamic molecules are less of an issue when the molecule is *permanently* encapsulated inside the fiberless radiative effector. Fourth, targeting the fiberless radiative effector is orders of magnitude more efficient (*i.e.*, the binding to the antigen has an equilibrium distribution constant that is orders of magnitude larger compared to isolated, targeted photodynamic molecules). Fifth, unlike traditional photodynamic therapy, magnetic signaling/targeting and magnetocatalysis via magnetic nanoparticles is possible with the fiberless radiative effectors of the present invention. Sixth, non-traditional methods of photodynamic therapy can be utilized with the fiberless radiative effectors of the present invention. For instance, x-rays or gamma-rays can be used for excitation, instead of standard light sources (*e.g.*, lasers). This is demonstrated with x-ray transducing materials (*e.g.*, polystyrene based nanoscintillators), that transfer the energy to the photoactive molecule (one absorbed x-ray photon gives the equivalent of 500 absorbed visible

photons). The very same principle works for gamma-rays. Even the visible photon absorption and quantum efficiency can be much improved by supra- or super-molecular antenna systems, in analogy to the photosynthetic antenna in green plants. The fiberless radiative effector core thus serves as an efficient amplification system. This makes it possible to use significantly reduced doses of irradiation, thus minimizing side effects. Additionally, there is practically no limit on treatable tumor depth with the use of x-rays or gamma rays for photodynamic excitation.

Furthermore, the use of photodynamic therapy for treatment of pathogen exposure is facilitated by the use of fiberless radiative effectors of the present invention. Specifically, it is contemplated that the therapeutic index of photodynamic compounds for this use will be increased by several orders of magnitude. It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is contemplated that the selectivity (*i.e.*, specificity) of fiberless radiative effectors results in attachment predominately to invading bacteria, which generally take up less than 10^{-8} of the body's weight (based on less than 10^4 bacteria/ml blood). Therefore, one needs only about one in 10^8 of the photodynamic compound dose usually administered (intravenously). To be conservative, this fraction may be increased to about 10^{-6}, and in order to reduce by a factor of 1000 the needed irradiation time (*i.e.*, from about 10^{-3} to 1 second), it can be increased further to 10^{-3} (*i.e.*, 0.1 mg total dye compared to traditional doses of 100 mg or higher). It is contemplated that this eliminates undesired side effects. In contrast to individual photodynamic compounds, in some embodiments fiberless radiative effectors do not enter cells, except possibly macrophage cells (*i.e.*, when the "cloaking" fails). At the projected short irradiation times, one single macrophage will have to incorporate thousands of fiberless radiative effectors to be photodamaged. Even if the majority of the fiberless radiative effectors are taken up by macrophages, the integrated damage to the macrophage population will be negligible. As long as one ppm of the fiberless radiative effectors escape the macrophages and find their target, most bacteria will be photoinactivated.

Methods for preparing basic fiberless radiative effectors are described below, followed by descriptions of toxic agents, cloaking materials, and molecular recognition elements that may

be utilized with the fiberless radiative effectors. Uses of the fiberless radiative effectors are then discussed in detail.

I. Matrix Materials for Fiberless Radiative Effectors

The fiberless radiative effectors of the present invention are preferably small particles (*i.e.*, fiberless) with a core matrix. The present invention contemplates the fabrication of the fiberless radiative effector from matrix materials in which toxic agents are incorporated or embedded, to which molecular recognition elements are attached, and that are cloaked or placed in a cloaking material. It is also desirable that the matrix material allow the diffusion of free radicals and other small molecules into and out of the particle the fiberless radiative effector. The matrix material of the present invention is also biologically compatible (*i.e.*, non-toxic to biological systems) and should allows energy transduction (*i.e.*, allow the passage of photons through the material).

The present invention is not limited to a particular matrix material. Indeed, a variety of matrix materials are contemplated. In some embodiments, a polymeric matrix (*e.g.*, polyacrylamide or poly(vinyl chloride)) is utilized, while in other embodiments a glass matrix is utilized (*e.g.*, sol-gel). In some embodiments of the present invention, the fiberless radiative effectors are finely ground or formed particles, with sizes ranging from 1 nm to 5 μm, preferably from about 40 to 400 nm. The matrices can be hydrophobic (*e.g.*, decyl-methacrylate), hydrophilic (*e.g.*, polyacrylamide), or biodegradable (*e.g.*, poly(lactic acid)).

In some embodiments, fiberless radiative effectors are fabricated from polyacrylamide. In some preferred embodiments, about 27% acrylamide and about 3% N,N-methylene-bis(acrylamide) are combined in a buffer (*e.g.*, 0.1M phosphate buffer, pH 6.5). About one ml of this solution is then added to a solution containing about 20 ml hexane, 1.8 mM sodium dioctyl sulfosuccinate, and about 4.24 mM Brij 30 (*i.e.*, 4 lauryl ether). The solution is then stirred under nitrogen for 20 minutes while cooling in an ice bath. The polymerization is initiated with about 24 μl of a 10% ammonium persulfate solution and about 12 μl N.N,N',N'-tetraethyldiethylenetriamine (TEMED). The solution is then stirred at room

temperature for about 2 hours. Hexane is removed by rotary evaporation, and the probes are rinsed free of surfactant with ethanol. This procedure yields particles of about 20 to 200 nm.

In other embodiments, fiberless radiative effectors are fabricated from poly(vinyl chloride). In some preferred embodiments, the poly(vinyl chloride) (*e.g.*, about 33 wt %) is combined with a plasticizer (*e.g.*, about 66 wt %) and dissolved in a solvent (*e.g.*, about 200 mg of poly(vinyl chloride)/plasticizer mixture is added to 5 ml freshly distilled tetrahydrofuran (THF)). The solution is then coated onto polystyrene spheres of the desired size (*e.g.*, about 10-1000 nm), and the coated spheres ground in liquid nitrogen.

In still other embodiments, the fiberless radiative effectors are fabricated from decy-methacrylate. In some preferred embodiments, a polymerization solution is made by combining about 55 mg of decyl-methacrylate and about 75 mg of hexanediol dimethacrylate. The solution is then washed three times with 5% sodium hydroxide solution and three times with water. About 100 mg of dioctyl sebacate (DOS), 5 mg of benzophenone, and 2.5 mg of benzoyl peroxide are added to the washed monomer. This solution is then added to 1 ml of water and sonicated for 1 hour until uniform. The spheres are then purged with nitrogen for 20 min, and polymerized with a UV lamp for 15 min.

In still other embodiments, the fiberless radiative effectors are fabricated using a sol-gel procedure. In some preferred embodiments, a polymerization solution is prepared comprising about 1.5 g sodium bis(2-ethylhexyl) sulfosuccinate (AOT), about 3 g Brij 30, about 9 ml hexane, about 4.5 ml tetraethylorthosilicate (TEOS), and about 250 ml NH_3H_2O. The solution is allowed to react for 18 hrs, with stirring, at room temperature.

In some preferred embodiments, a co-polymer with functionalized groups or otherwise reactive groups (*e.g.*, amine groups) is included during the fabrication of the fiberless radiative effector. The co-polymer allows the convenient attachment of molecular recognition elements and other compounds (*e.g.*, cloaking materials, biotin) to the fiberless radiative effector. Useful co-polymers include, but are not limited to, (N-(3-Aminopropyl)methacrylamide hydrochloride) (APMA), acryloamidodextran, aminomethylstyrene, 2-hydroxyethyl acrylate, 2-hydroxymethacrylate, 4-hydroxybutyl acrylate

(*See e.g.*, Daubresse *et al.*, J. Pharm. Pharmacol. 45(12):1018-23 [1993]; Noguchi *et al.*, J. Biomed. Mat. Res. 39(4):621-9 [1998]).

II. Toxic Agents for Incorporation Into Fiberless Radiative Effectors

A variety of toxic agents are incorporated into the fiberless radiative effector. The toxic agents may themselves be toxic, or they may act in concert with another compound, material, or external stimulus to produce a toxic compound, material, or molecule. The toxic agents are preferably incorporated into the fiberless radiative effectors by inclusion in the polymerization mixtures described above. In some embodiments, the toxic agents include, but are not limited to, photodynamic or radiodynamic compounds. Some preferred photodynamic compounds include, but are not limited to, those that can participate in a type II photochemical reaction:

$$PS + h\nu \rightarrow PS^*(1)$$

$$PS^*(1) \rightarrow PS^*(3)$$

$$PS^*(3) + O_2 \rightarrow PS + {}^*O_2$$

$${}^*O_2 + T \rightarrow \text{cytotoxity}$$

where PS = photosenstizer, PS*(1) = excited singlet state of PS, PS*(3) = excited triplet state of PS, hν = light quantum, *O2 = excited singlet state of oxygen, and T = biological target. Other photodynamic compounds useful in the present invention include those that cause cytotoxity by a different mechanism than singlet oxygen production (*e.g.*, copper benzochlorin, Selman, *et al.*, Photochem. Photobiol., 57:681-85 [1993], incorporated herein by reference). Examples of photodynamic compounds that find use in the present invention include, but are not limited to Photofrin 2, phtalocyanins (*See e.g.*, Brasseur *et al.*, Photochem. Photobiol., 47:705-11 [1988]), benzoporphyrin, tetrahydroxyphenylporphyrins, naphtalocyanines (*See e.g.*, Firey and Rodgers, Photochem. Photobiol., 45:535-38 [1987]), sapphyrins (Sessler *et al.*, Proc. SPIE, 1426:318-29 [1991]), porphinones (Chang *et al.*, Proc. SPIE, 1203:281-86 [1990]), tin etiopurpurin, ether substituted porphyrins (Pandey *et al.*, Photochem. Photobiol., 53:65-72 [1991]), and cationic dyes such as the phenoxazines (*See e.g.*, Cincotta *et al.*, SPIE Proc., 1203:202-10 [1990]).

In other embodiments, toxic agents that directly produce free radicals (*i.e.*, do not produce singlet oxygen) are incorporated into the fiberless radiative effectors during polymerization. This approach allows for larger and longer lived fiberless radiative effectors and will not be limited by local oxygen supplies. Such toxic agents include, but are not limited to 2-methyl-4-nitro-quinoline-N-oxide (Aldrich) and 4,4-dinitro-(2,2) bipyridinyl- N,N dioxide (Aldrich), which produce hydroxyl radicals when illuminated with 360-400 nm light (Botchway *et al.*, Photochem. Photobiol. 67(7):635-40 [1998]); malachite green and isofuran blue (Molecular Probes), which produce hydroxyl radicals upon stimulation with about 630 nm light (Jay *et al.*, PNAS 91:2659 [1994]; Haugland, Handbook of Fluorescent Probes and Research Chemicals, 6th ed., Molecular Probes, Eugene, OR [1994]); potassium nitrosylpentachlororuthenate (Molecular Probes) (abs = 516 nm), Roussin's black salt and Roussin's red salt (abs 313-546 nm), serve as sources of NO which is toxic to cells (Murphy *et al.*, Neuropharm. 33:1375-85 [1994]; Bourassa *et al.*, JACS 119:2853-60 [1997]); and other photolytic nitric oxide and hydroxyl donors (De Leo and Ford, JACS 121:1980-81 [1999]).

In still other embodiments, the toxic agents are preferably radiodynamic compounds. Fiberless radiative effectors incorporating radiodynamic compounds preferably comprise a polystyrene or polystyrene like matrix (*e.g.*, poly(styrene)-poly(vinylpyridine) that serves as a molecular antennae (*See e.g.*, Kokotov *et al.*, Photochem. Photobiol. 59(3):385-87 [1994], incorporated herein by reference). Additionally, the radiodynamic fiberless radiative effectors preferably include a sensitizer such as a scintillating compound (*e.g.*, NaI-125, 2,5-diphenyloxazole (PPO); 2-(4-biphenyl)-6-phenylbenzoxazole; 2,5-bis-(5'-tert-butylbenzoxazoyl-[2'])thiophene; 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole; 1,6-diphenyl-1,3,5-hexatriene; trans-p,p'-diphenylstilbene; 2-(1-naphthyl)-5-phenyloxazole; 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole; p-terphenyl; and 1,1,4,4-tetraphenyl-1,3-butadiene) and a porphyrin containing compound (*e.g.*, hematoporphyrin or Photofrin II). It is not intended that the present invention be limited to a particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is believed that the molecular antennae is excited by x-ray or gamma ray photons to produce excitons. The excitons are then transferred to the sensitizer and then to the

porphyrin or other photodynamic compound. This transfer of energy results in the production of singlet oxygen and other free radicals as described above.

It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is believed that the fiberless radiative effectors of the present invention cause cell death or apoptosis due to the generation of singlet oxygen and other free radicals (*e.g.*, superoxide and hydroxyl radical) that diffuse from the fiberless radiative effector to interact with the biological target. With respect to tumors, it is thought that the tumors are initially sublethally damaged, and then die when subsequent vascular collapse results in tumor hypoxia.

III. Other Materials for Inclusion in Fiberless Radiative Effectors

In some embodiments, it is contemplated that the fiberless radiative effector further comprise a radioactive element or compound that emits gamma rays (*e.g.*, ^{111}In-oxine, ^{59}Fe, ^{67}Cu, ^{125}I, ^{99}Te (Technetium), and ^{51}Cr) or elements or compounds that emit beta particles (*e.g.*, ^{32}P, ^{3}H, ^{35}S, ^{14}C, ^{90}Y). Fiberless radiative effectors containing such elements are utilized as a discreet energy source for the excitation of a separate fiberless radiative effector that includes a radiodynamic or photodynamic compound. In some embodiments, the fiberless radiative effector also includes a scintillating material so that photons are produced as a result of the emission of the beta particles or gamma rays. Some of these photons are transmitted to the fiberless radiative effector containing a photodynamic dye so that singlet oxygen and other radicals are produced.

In other embodiments of the present invention, the radioactive element or compound is preferably the toxic agent. In some embodiments, fiberless radiative effectors containing a radioactive element are used for internal radiation therapy (*See e.g.*, Yan *et al.*, Cancer 72(11):3210-15 [1993]; Ariel *et al.*, J. Surg. Oncol. 20:151-6 [1982]; Ehrhardt and Day, Nucl. Med. Biol. 14(3):233-42 [1987]; each of which is incorporated herein by reference).

In other embodiments of the present invention, the fiberless radiative effector preferably includes dendrimeric molecules (*See e.g.*, Swallen *et al.*, J. Luminescence 76&77:193-96

[1998]; Bar-Haim *et al.*, JACS 119:6197-98 [1997], both incorporated herein by reference). Dendrimeric molecules are composed of repeating units (*e.g.*, phenylacetylene), growing exponentially in successively larger generations out from a central core. Dendrimers are specified by three main characteristics: the basic chemical building elements (*e.g.*, phenylacetylene); the branching (*i.e.*, coordination) number, Z, at each node, and the number of generations. Each successive generation contains a factor of (Z-1) more elements than the previous one, and thus the total number of elements increases exponentially with generation number. It is not intended that the present invention be limited to a particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is believed that the dendrimer molecules act as antennas that funnel absorbed light to the photodynamic compound. In some embodiments of the present invention, the incorporation of dendrimer molecules into the fiberless radiative effectors increases photostability.

In some embodiments of the present invention, the fiberless radiative effectors preferably includes a protein (*e.g.*, luciferase) that catalyzes a reaction that produces a photon (i.e., bioluminescence). Many different luciferases are incorporated into the fiberless radiative effectors of the present invention, including, but not limited to, bacterial luciferase (U.S. Pat. No. 4,548,994), *Photinus pyralis* luciferase (U.S. Pat. Nos. 5,670,356 and 5,674,713), *Renilla reniformus* luciferase, *Pyrophorus plagiophthalamus* luciferase, *Luciola cruciata* luciferase (Masuda *et al.*, Gene 77:265-70 [1989]), *Luciola lateralis* luciferase (Tatsumi *et al.*, Biochim. Biophys. Acta 1131:161-65 [1992]), and *Latia neritoides* luciferase. In some preferred embodiments, luciferase is incorporated into sol-gel or polyacrylamide fiberless radiative effectors. The luciferase-containing fiberless radiative effectors find use in the present invention for labelling biological targets so that the target is visualized following excitation with light of the appropriate wavelength in the presence of appropriate substrates (*e.g.*, luciferin and ATP).

In other embodiments of the present invention, the fiberless radiative effectors preferably include an antioxidant or antifade agent. Antioxidants are preferably only included in fiberless radiative effectors not containing a photodynamic or radiodynamic compound, as the

antioxidant would quench singlet oxygen and free radical production. However, the inclusion of antioxidants in fiberless radiative effectors containing, for instance, radioactive elements, is desirable so that the fiberless radiative effector is protected from oxidation. Suitable antioxidants or antifade agents include, but are not limited to ascorbic acid, vitamin C, E, beta-carotene and their derivitives and other dietary antioxidants, phenylalanine, azide, p-phenylenediamine, n-propylgallate, diazabicyclo[2,2,2]octane, and the commercial reagents SlowFade and ProLong (Molecular Probes, Eugene OR).

IV. Cloaking Materials for Fiberless Radiative Effectors

A variety of cloaking materials or agents find use with the fiberless radiative effectors of the present invention, including, but not limited to poly(ethylene glycol), liposomes, poly(ethylene glycol) coated liposomes, oxidized dextran, and red blood cell ghosts. Preferably, the cloaking material protects the fiberless radiative effector from recognition by the immune system and circulatory reticulo-endothelial system (RES). This results in both increased delivery of the fiberless radiative effector to the intended biological target and reduced toxicity to other body tissues.

Many suitable poly(ethylene glycol)(PEG) based materials are known (*See e.g.*, Zalipsky, Bioconjug. Chem. 6:150-65 [1995]; Coombes *et al.*, Biomaterials 18:1153-61 [1997]; Vandorpe *et al.*, Biomaterials 18:1147-52 [1997]; and Zalipsky *et al.*, J. Cont. Release 39:152-61 [1996], each incorporated herein by reference). The present invention contemplates such PEG-based materials as cloaking agents. In some preferred embodiments, derivatized or functionalized PEG (*e.g.*, amino-PEG, sulfonate esters of PEG, hydrazido-PEG, and carboxyl-PEG) is utilized to cloak the fiberless radiative effector. Other cloaking materials include, but are not limited to poloxamer and poloxamine block co-polymers (*See e.g.*, Torchilin, J. Microencapsulation 15(1) 1-19 [1998]) such as poly(phosphazene)-poly(ethylene oxide)(Vandorpe *et al., supra*), and poly(ethylene oxide)-poly(propylene oxide)(Coombes *et al., supra*).

In some embodiments, the functionalized PEG or other cloaking material is covalently bound to the polymer or copolymer (*e.g.*, APMA) forming the fiberless radiative effector by

utilizing the appropriate homobifunctional (*e.g.*, disuccinimidyl suberate, bis(sulfosuccinymidyl)suberate, disuccinimydal glutarate, dimethyladipimidate-2HCl, or dimethylpimelimidate-2HCl) or heterobifunctional reagent (*e.g.*, succinimydyl 3-(2-pyridyldithio)propionate (SPDP); succinimidyl trans-4-(N-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC); succinimidyl (acetylthio)acetate (SATA); 4-[(succinimydyloxy)carboxyl]-α-methyl-α-(2-pyridyldithio)toluene (SMPT); succinimidyl 4-[[(iodoacetyl0amino]methyl]-cyclohexane-1-carboxylate (SIAC); m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), succinimydyl 4-(p-maleimdophenyl)-butyrate (SMPB) and succinimidyl *p*-azidobenzoate (SAB)). In other embodiments, the fiberless radiative effectors are first silanized (*e.g.*, with 3-(trimethoxysilyl)propyl methacrylate, Barker *et al.*, Anal. Chem. 70:971-76 [1998]; Barker *et al.*, Anal. Chem. 69:990-95 [1997], both incorporated herein by reference). Functionalized PEG or other cloaking materials can then be covalently bound to the silanized fiberless radiative effector.

In still other embodiments, the cloaking material is a liposome. In some preferred embodiments, a plurality of fiberless radiative effectors are contained within the liposome. A variety of methods are known for producing liposomes with good stability in the circulatory system. In some embodiments, liposomes are sterically stabilized by incorporation of the end group functionalized PEG-lipid derivative pyridylthiopropionoylamino-PEG-distearoylphosphatidylethanolamine (PDP-PEG-DSPE)(*See e.g.*, Allen *et al.*, Biochim. Biophys. Acta 1237:99-108 [1995], incorporated herein by reference.). In other embodiments, liposomes are fabricated that are sterically stabilized with disteroyl-N-(3-carboxypropionoyl poly(ethylene glycol) succinyl)phosphatidylethanolamine (DSPE-PEG-COOH)(*See e.g.*, Maruyama *et al.*, Biochim. Biophys. Acta 1234:74-80 [1995], incorporated herein by reference). In some embodiments, molecular recognition elements are covalently attached to the stabilized liposome.

In other embodiments, the cloaking material is a red blood cell ghost. In some preferred embodiments, a plurality of fiberless radiative effectors are contained within the red blood cell ghosts (*See e.g.*, Matteo *et al.*, Am. J. Obstet. Gynecol. 178(2):402-08 [1998]; Heinz and Hoffman, J. Cell Comp. Physiol. 65:31-44 [1965], both incorporated herein by reference). An

advantage of red blood cell ghosts is that integral membrane proteins remain in place and can be used as a convenient means for covalent attachment of molecular recognition elements.

V. Molecular Recognition Elements for Fiberless Radiative Effectors

In other embodiments of the present invention, a molecular recognition element is attached, fixed or conjugated to the fiberless radiative effector so that it can be targeted to a particular biological target. It is contemplated that the fiberless radiative effectors of the present invention are targeted via the molecular recognition element to a variety of biological targets, including, but not limited to, tumor cells, bacteria, viruses, cell surface proteins, cell surface receptors, cell surface polysaccharides, extracellular matrix proteins, intracellular proteins and intracellular nucleic acids. The present invention is not limited to any particular molecular recognition element. Indeed a variety of molecular recognition elements are contemplated. Examples of molecular recognition elements that find use in the present invention include, but are not limited to, nucleic acids (*e.g.*, RNA and DNA), polypeptides (*e.g.*, receptor ligands, signal peptides, avidin, Protein A, antigen binding proteins), polysaccharides, biotin, hydrophobic groups, hydrophilic groups, drugs and any organic molecules that bind to receptors. It is contemplated that the fiberless radiative effectors of the present invention display (*i.e.*, be conjugated to) one, two, or a variety of molecular recognition elements.

Utilization of more than one molecular recognition element in a fiberless radiative effector allows multiple biological targets to be targeted. Multiple molecular recognition elements also allows the fiberless radiative effectors to be utilized in a "stacking" approach wherein a first fiberless radiative effector (*e.g.*, comprising a radiodynamic compound) is targeted to a biological target, and a second fiberless radiative effector (*e.g.*, comprising a gamma-ray emitter and avidin as a molecular recognition element) is targeted to a second molecular recognition element (*e.g.*, biotin) on the first fiberless radiative effector.

A. Peptides that Specifically Target Tumor Cells

In some preferred embodiments, the fiberless radiative effector molecular recognition elements comprise peptides that bind specifically to tumor blood vessels (*See e.g.*, Arap *et al.*, Science 279:377-80 [1998], incorporated herein by reference). These peptides include, but are not limited to, peptides containing the RGD (Arg-Gly-Asp) motif (*e.g.*, CDCRGDCFC; SEQ ID NO:1), the NGR (Asn-Gly-Arg) motif (*e.g.*, CNGRCVSGCAGRC; SEQ ID NO:2), or the GSL (Gly-Ser-Leu; SEQ ID NO:3) motif. These peptides and conjugates containing these peptides selectively bind to various tumors, including, but not limited to, breast carcinomas, Karposi's sarcoma, and melanoma. It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is believed that these peptides are ligands for integrins and growth factor receptors that are absent or barely detectable in established blood vessels. In some preferred embodiments, the peptide is preferably produced using chemical synthesis methods. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*See e.g.*, Creighton (1983) Proteins Structures And Molecular Principles, W H Freeman and Co, New York N.Y.). In other embodiments of the present invention, the composition of the synthetic peptides is confirmed by amino acid analysis or sequencing (*See e.g.*, Creighton, *supra*).

B. Peptides that Specifically Target Gliomas

In some preferred embodiments, the fiberless radiative effector molecular recognition elements comprise peptides that specifically bind to glioma cells (*See e.g.*, Debinski *et al.*, Nature Biotech. 16:449-53 [1998]; Debinski *et al.*, J. Biol. Chem. 270(28):16775-80 [1995]; and Debinski *et al.*, J. Biol. Chem. 271(37):22428-33 [1996], each incorporated herein by reference). In some embodiments, the present invention contemplates fiberless radiative effectors comprising IL13 or one of its variants so that the fiberless radiative effector will bind to IL13 binding sites in glioma cells.

Human high-grade gliomas are uniquely enriched in IL13 binding sites. Many of the established brain tumor cell lines, primarily malignant gliomas, over-express hIL13 binding sites. Human malignant glioma cell lines express high number, up to 30,000, binding sites for hIL13 per cell. Of interest, glioblastoma multiforme (GBM) explant cells showed an extraordinary high number of hIL13 binding sites, up to 500,000 per cell. The binding of hIL13 is not neutralized by hIL4 on an array of established human glioma cell lines that includes U-251 MG, U-373 MG, DBTRG MG, Hs-683, U-87 MG, SNB-19, and A-172 cells.

hIL13 (SEQ ID NO:4, *see* Fig. 7) can be engineered to increase its specific targeting of high-grade gliomas. The pattern for IL13- and IL4R sharing on normal cells requires IL13 to bind hIL4R This is confirmed by the fact that hIL13 binding is always fully competed by hIL4. The recently proposed model for this hIL13R suggests that the shared hIL13/4R is heterodimeric. This scenario would imply that hIL13 may contain at least two receptor-binding sites, each recognizing a respective subunit of the receptor. The engineered hIL13 variants (*e.g.*, hIL13.E13K (SEQ ID NO:5) or hIL13.E13Y (SEQ ID NO:6), *see* Fig. 5) are deprived of cell signaling abilities (Debinski *et al.*, [1998], *supra*). This is desirable because interaction with physiological systems contributes prominently to the dose-limiting toxicity of some biological therapeutics (*e.g.*, cytokines). Significantly, the molecule of hIL13 appears not to be sensitive to a variety of genetically engineered modifications and these variants can be produced in large quantities. It is thus possible to divert the molecule of hIL13 from its physiological receptor and make it a non-signaling compound, while its affinity toward the HGG-associated receptor remains intact or is increased. Such forms of IL13 can serve as rationally designed vectors for variety of imaging and therapeutic approaches of HGG.

Given the grim prognosis following the identification of an intracranial malignancy, any strategy for the pre-, intra- or post-operative identification and removal of cancer cells is a significant improvement. Recent discovery of the expression of IL-13 receptors on the surface of all of the malignancies of glial origin provides a novel strategy for the accumulation and retention of fiberless radiative effectors within CNS cancers. The high-grade glioma-associated receptor for IL13 used in the present invention is much more specific than the receptor for either transferrin or EGF, since its normal tissue presence is negligible or

null. Also, the over-expression of the IL13R in gliomas is much more frequent and homogenous than that for EGF.

In some embodiments, nucleic acids (*e.g.*, SEQ ID NO:7) encoding IL13 fragments, fusion proteins or functional equivalents or variants (*e.g.*, hIL13.E13K or hIL13.E13Y) thereof are cloned into an appropriate expression vector, expressed and purified (*e.g.*, preferably as described in Debinski *et al.*, Nature Biotech. 16:449-53 [1998]; Debinski *et al.*, J. Biol. Chem. 270(28):16775-80 [1995]; and Debinski *et al.*, J. Biol. Chem. 271(37):22428-33 [1996], each of which is incorporated by reference). In other embodiments of the present invention, vectors include, but are not limited to, chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available. Such vectors include, but are not limited to, the following vectors: 1) Bacterial - pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pbluescript SK, pBSKS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); and 2) Eukaryotic - pWLNEO, pSV2CAT, pOG44, PXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). Any other plasmid or vector can be used as long as they are replicable and viable in the host. In some preferred embodiments of the present invention, mammalian expression vectors comprise an origin of replication, a suitable promoter and enhancer, and any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. In other embodiments, DNA sequences derived from the SV40 splice, and polyadenylation sites are used to provide the required nontranscribed genetic elements.

In other embodiments, the IL13 peptide or variant thereof is expressed in a host cell. In some embodiments of the present invention, the host cell is a higher eukaryotic cell (*e.g.*, a mammalian or insect cell). In other embodiments of the present invention, the host cell is a lower eukaryotic cell (*e.g.*, a yeast cell). In still other embodiments of the present invention, the host cell can be a prokaryotic cell (*e.g.*, a bacterial cell). Specific examples of host cells

include, but are not limited to, *Escherrichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, as well as, *Saccharomycees cerivisiae*, *Schizosaccharomycees pombe*, *Drosophila* S2 cells, *Spodoptera* Sf9 cells, Chinese Hamster Ovary (CHO) cells, COS-7 lines of monkey kidney fibroblasts, (Gluzman, Cell, 23:175 [1981]), C127, 3T3, HeLa and BHK cell lines.

In some embodiments of the present invention, IL13 or variants thereof are recovered or purified from recombinant cell cultures by methods including, but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. In other embodiments of the present invention, protein refolding steps are used, as necessary, in completing configuration of the mature protein. In still other embodiments of the present invention, high performance liquid chromatography (HPLC) is employed for final purification steps.

Some embodiments of the present invention provide polynucleotides having the coding sequence fused in frame to a marker sequence that allows for purification of the polypeptide of the present invention. A non-limiting example of a marker sequence is a hexahistidine tag that is supplied by a vector, preferably a pQE-9 vector, that provides for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host (*e.g.*, COS-7 cells) is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, *et al.*, Cell, 37:767 [1984]).

D. Signal Peptides As Molecular Recognition Elements

In some embodiments of the present invention, the molecular recognition element is preferably a signal peptide. These peptides are chemically synthesized or cloned, expressed and purified as described above. The signal peptides are used to target the fiberless radiative effector to a discreet region within a cell. In some embodiments, fiberless radiative effectors

with signal peptides as molecular recognition elements are delivered to cells via targeted liposomes.

Specific amino acid sequences in proteins are responsible for targeting the polypeptide into cellular organelles and compartments. In some embodiments, the signal peptides direct protein import into mitochondria. In further embodiments, the molecular recognition element is preferably: NH- Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-COOH (SEQ ID NO:8). It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is contemplated that this peptide forms an amphipathic helix that associates with mitochondrial membranes sites of protein import. This allows the peptide-fiberless radiative complex to attach to mitochondrial membranes. It is unlikely that the complex will be internalized, since there are few pores of nm size on intact mitochondria.

In still other embodiments, the following nuclear localization signal is utilized: NH-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-COOH (SEQ ID NO:9). In another embodiment, SNAP-25, is utilized to deliver fiberless radiative effectors to the presynaptic region of neuronal cells. It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it is contemplated that SNAP-25 is one of the prototypic v-SNARE proteins. SNAP-25 localizes specifically to the presynaptic terminals of neuronal cells and PC-12 cells in culture. It is not known which portion of the peptide is responsible for sorting to the presynaptic terminal. However, during cellular processing of the peptide, SNAP-25 becomes palmitoylated at a central Cys-quartet. These palmitylated groups help anchor the protein in the presynpatic membrane. SNAP-25 associates with syntaxin, and ultimately, with the entire vesicular fusion machinery in a calcium-activated presynaptic terminal.

E. Antibodies as Molecular Recognition Elements

In some embodiments of the present invention, the molecular recognition elements are preferably antigen binding proteins or antibodies. Antibodies can be generated to allow for

the targeting of antigens or immunogens (*e.g.*, tumor , tissue or pathogen specific antigens) on various biological targets (*e.g.*, pathogens, tumor cells, normal tissue). Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library.

In some preferred embodiments, the antibodies recognize tumor specific epitopes (e.g., TAG-72 (Kjeldsen *et al.*, Cancer Res. 48:2214-2220 [1988]; U.S. Pat. Nos. 5,892,020; 5,892,019; and 5,512,443); human carcinoma antigen (U.S. Pat. Nos. 5,693,763; 5,545,530; and 5,808,005); TP1 and TP3 antigens from osteocarcinoma cells (U.S. Pat. No. 5,855,866); Thomsen-Friedenreich (TF) antigen from adenocarcinoma cells (U.S. Pat. No. 5,110,911); "KC-4 antigen" from human prostrate adenocarcinoma (U.S. Pat. Nos. 4,708,930 and 4,743,543); a human colorectal cancer antigen (U.S. Pat. No. 4,921,789); CA125 antigen from cystadenocarcinoma (U.S. Pat. No. 4,921,790); DF3 antigen from human breast carcinoma (U.S. Pat. Nos. 4,963,484 and 5,053,489); a human breast tumor antigen (U.S. Pat. No. 4,939,240); p97 antigen of human melanoma (U.S. Pat. No. 4,918,164); carcinoma or orosomucoid-related antigen (CORA)(U.S. Pat. No. 4,914,021); a human pulmonary carcinoma antigen that reacts with human squamous cell lung carcinoma but not with human small cell lung carcinoma (U.S. Pat. No. 4,892,935); T and Tn haptens in glycoproteins of human breast carcinoma (Springer *et al.*, Carbohydr. Res. 178:271-292 [1988]), MSA breast carcinoma glycoprotein termed (Tjandra *et al.*, Br. J. Surg. 75:811-817 [1988]); MFGM breast carcinoma antigen (Ishida *et al.*, Tumor Biol. 10:12-22 [1989]); DU-PAN-2 pancreatic carcinoma antigen (Lan *et al.*, Cancer Res. 45:305-310 [1985]); CA125 ovarian carcinoma antigen (Hanisch *et al.*, Carbohydr. Res. 178:29-47 [1988]); YH206 lung carcinoma antigen (Hinoda *et al.*, (1988) Cancer J. 42:653-658 [1988]). Each of the foregoing references are specifically incorporated herein by reference.

In other preferred embodiments, the antibodies recognize specific pathogens (*e.g.*, *Legionella peomophilia*, *Mycobacterium tuberculosis*, *Clostridium tetani*, *Hemophilus influenzae*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Bacillus anthracis*, *Vibrio cholerae*, *Borrelia burgdorferi*, *Cornebacterium diphtheria*, *Staphylococcus aureus*, human papilloma virus, human immunodeficiency virus, rubella virus, polio virus, and the like).

Various procedures known in the art are used for the production of polyclonal antibodies. For the production of antibody, various host animals can be immunized by injection with the peptide corresponding to the desired epitope including but not limited to rabbits, mice, rats, sheep, goats, etc. In a preferred embodiment, the peptide is conjugated to an immunogenic carrier (*e.g.*, diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants are used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides,oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (*See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, NY). These include, but are not limited to, the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, Nature 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (*See e.g.*, Kozbor *et al.* Immunol. Today 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, *in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (*See e.g.*, PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote *et al.*, Proc. Natl. Acad. Sci. U.S.A.80:2026-2030 [1983]) or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, *in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96 [1985]).

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; herein incorporated by reference) can be adapted to produce specific single chain antibodies. An additional embodiment of the invention utilizes

the techniques described for the construction of Fab expression libraries (Huse *et al.*, Science 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')2 fragment that can be produced by pepsin digestion of the antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western Blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.).

F. Other Molecular Recognition Elements

The molecular recognition elements of the fiberless radiative effectors of the present invention may recognize a variety of other epitopes on biological targets (*e.g.*, pathogens, tumor cells, normal tissues). In some embodiments, molecular recognition elements are incorporated to recognize, target or detect a variety of pathogenic organisms including, but not limited to, sialic acid to target HIV (Wies *et al.*, Nature 333: 426 [1988]), influenza (White *et al.*, Cell 56: 725 [1989]), *Chlamydia* (Infect. Imm. 57: 2378 [1989]), *Neisseria meningitidis*, *Streptococcus suis*, *Salmonella*, mumps, newcastle, and various viruses, including reovirus, Sendai virus, and myxovirus; and 9-OAC sialic acid to target coronavirus, encephalomyelitis virus, and rotavirus; non-sialic acid glycoproteins to detect cytomegalovirus (Virology 176: 337 [1990]) and measles virus (Virology 172: 386 [1989]); CD4 (Khatzman *et al.*, Nature 312: 763 [1985]), vasoactive intestinal peptide (Sacerdote *et al.*, J. of Neuroscience Research

18: 102 [1987]), and peptide T (Ruff *et al.*, FEBS Letters 211: 17 [1987]) to target HIV; epidermal growth factor to target vaccinia (Epstein *et al.*, Nature 318: 663 [1985]); acetylcholine receptor to target rabies (Lentz *et al.*, Science 215: 182 [1982]); Cd3 complement receptor to target Epstein-Barr virus (Carel *et al.*, J. Biol. Chem. 265: 12293 [1990]); β-adrenergic receptor to target reovirus (Co *et al.*, Proc. Natl. Acad. Sci. 82: 1494 [1985]); ICAM-1 (Marlin *et al.*, Nature 344: 70 [1990]), N-CAM, and myelin-associated glycoprotein MAb (Shephey *et al.*, Proc. Natl. Acad. Sci. 85: 7743 [1988]) to target rhinovirus; polio virus receptor to target polio virus (Mendelsohn *et al.*, Cell 56: 855 [1989]); fibroblast growth factor receptor to target herpes virus (Kaner *et al.*, Science 248: 1410 [1990]); oligomannose to target *Escherichia coli*; ganglioside G_{M1} to target *Neisseria meningitidis*; and antibodies to detect a broad variety of pathogens (*e.g.*, *Neisseria gonorrhoeae*, *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae*, and *V. alginolyticus*).

G. Use of Nucleic Acids as Molecular Recognition Elements

In some embodiments of the present invention, the molecular recognition elements are preferably nucleic acids (*e.g.*, RNA or DNA). In some embodiments, the nucleic acid molecular recognition elements are designed to hybridize by base pairing to a particular nucleic acid (*e.g.*, chromosomal DNA, mRNA, or ribosomal RNA). In other embodiments, the nucleic acids bind a ligand or biological target. Nucleic acids that bind the following proteins have been identified: reverse transcriptase, Rev and Tat proteins of HIV (Tuerk *et al.*, Gene 137(1):33-9 [1993]); human nerve growth factor (Binkley *et al.*, Nuc. Acids Res. 23(16):3198-205 [1995]); and vascular endothelial growth factor (Jellinek *et al.*, Biochem. 83(34):10450-6 [1994]). Nucleic acids that bind ligands are preferably identified by the SELEX procedure (*See e.g.*, U.S. Pat. Nos. 5,475,096; 5,270,163; and 5,475,096; and in PCT publications WO 97/38134, WO 98/33941, and WO 99/07724, all of which are herein incorporated by reference), although many methods are known in the art.

VII. Attachment of Molecular Recognition Elements to Fiberless Radiative Effectors

A variety of methods are be used to attach, fix or conjugate the molecular recognition element or elements to fiberless radiative effectors. In some embodiments, as described above, a copolymer (*e.g.*, APMA) comprising a primary amine group is incorporated into the polymer matrix. The amine group serves as a convenient attachment point for molecular recognition elements. In some embodiments, the N-hydroxysuccinimide derivative of biotin is covalently linked to fiberless radiative effector and serves as a universal attachment point for other molecular recognition elements via an avidin linker. In other embodiments, the molecular recognition element is attached to the cloaking material. In still other embodiments, the molecular recognition element is attached to liposomes into which fiberless radiative effectors are loaded.

A. Biotinylated Fiberless Radiative Effectors

The fiberless radiative effectors of the present invention are covalently bound or attached to molecular recognition elements. In some preferred embodiments, fiberless radiative effectors that serve as universal acceptors for a variety of molecular recognition elements are provided. The fiberless radiative effectors comprise a material that serves as a convenient attachment point for a variety of molecular recognition elements. In some embodiments, the fiberless radiative effectors comprise biotin, which is attached by a variety of methods to either the fiberless radiative effector matrix material or the cloaking material. In other embodiments, the fiberless radiative effectors comprise protein A, anti-mouse IgG, anti-rabbit IgG, or some other protein that recognizes a variety of antibodies.

As described above, in some embodiments of the present invention, fiberless radiative effectors are synthesized that incorporate a copolymer that includes an amine group (*e.g.*, APMA). The amine groups provide convenient sites for the covalent attachment of N-hydroxysuccinimide derivatives of biotin. In some preferred embodiments, a N-hydroxysuccinimide derivative of biotin with a spacer arm, preferably a 10 to 15 carbon spacer arm, is utilized (*e.g.*, biotinamidocaproate N-hydroxysuccinimide ester, biotinamidocaproic acid 3-sulfo-N-hydroxysuccinimide ester, 6-(biotinamidocaproylamido)-caproic acid N-hydroxysuccinimide ester, sulfosuccinimidyl-6-(biotinamido) hexanoate, succinimidyl-6-(biotinamido) (*See e.g.*, Hofmann *et al.*, Biochem. 21:978 [1982]; Lantz and

Holmes, Biotechniques 18:58 [1995], Hyman *et al.*, Meth. Enzymol. 196:478 [1991]; Gretch *et al.*, Anal. Biochem. 163:270-77 [1987], each incorporated herein by reference). These biotinylated N-hydroxysuccinimide esters react with primary amines, especially aliphatic amines, in aqueous systems at pH 8.0 - 9.0. The aliphatic amide products that are formed are very stable. The inclusion of the spacer arm in the biotinylated N-hydroxysuccinimide esters reduces steric hindrance when binding several biotinylated molecules to one avidin complex.

In some embodiments, other reactive groups in co-polymers serve as attachment site for biotin *(e.g.*, biocytin hydrazide, biotin-LC-hydrazide, or biotin hydrazide are used to biotinylate aldehyde groups in carbohydrates; N-(6-[biotinamido]hexyl)-3'-(2'-pyridylthio)propionamide, 1-biotinamido-4-[4'(maleimidomethyl)-cyclohexane-carboxamido butane, 3-(N-maleimido-propionyl) biocytin (Bayer *et al.*, Anal. Biochem. 149:529-36 [1985]) or N-iodoacetyl-N-biotinyl hexylene diamine are used to biotinylate materials with thiol groups).

In other embodiments of the present invention, the cloaking agent is biotinylated with a suitable biotin derivative (*e.g.*, a N-hydroxysuccinimide derivative of biotin with a spacer arm is used to biotinylate fiberless radiative effectors cloaked with amino-PEG; biocytin hydrazide, biotin-LC-hydrazide, or biotin hydrazide are used to biotynylate fiberless radiative effectors cloaked with COOH-PEG). In some embodiments, biotinylated PEG is used as the cloaking material (*See e.g.*, Kaiser *et al.*, Bioconj. Chem. 8(4):545-51 [1997], incorporated herein by reference). In still other embodiments, the PEG coat of a liposome containing fiberless radiative effectors is biotinylated as above.

In some preferred embodiments of the present invention, peptide or protein molecular recognition elements (*e.g.*, antigen binding proteins, antibodies, RGD peptides, IL13 and its variants, or carbohydrates such as sialic acid) are also biotinylated by selecting an appropriate reagent as described above. In other preferred embodiments, nucleic acid molecular recognition elements are synthesized with biotinylated base (*See e.g.*, Leary *et al.*, PNAS 80: 4045-49 [1983]; Langer *et al.*, PNAS 78:6633-37 [1981]; Brigati *et al.* Virology 126: 32-50 [1983]), or biotinylated bases are added to 3' or 5' terminal base with a terminal transferase, *Taq* polymerase, or poly(A)polymerase (*See e.g.*, Sambrook *et al., Molecular Cloning: A*

Laboratory Manual, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989], incorporated herein by reference).

In still further embodiments of the present invention, the biotinylated molecular recognition element is linked to the biotinylated fiberless radiative effector (*i.e.*, biotinylated either directly or on the cloaking material) with avidin. Avidin is a glycoprotein found in egg white and contains four identical subunits having a combined molecular mass of 67 - 68 kD. Each subunit binds one molecule of biotin, therefore, combining the avidin, biotinylated fiberless radiative effector, and biotinylated molecular recognition element results in the formation of a fiberless radiative effector-biotin-avidin-biotin-molecular recognition element complex that binds to a biological target.

B. Direct Cross-Linking of Molecular Recognition Elements to Fiberless Radiative Effectors and Cloaking Materials

In some embodiments of the present invention, molecular recognition elements containing amine groups (*e.g.*, antigen binding proteins, antibodies, RGD peptides, or IL13 and its variants) are crosslinked with the fiberless radiative effector via a homobifunctional crosslinking agent or heterobifunctional cross-linking agent. In some preferred embodiments, the crosslinking agent includes a linker arm so that steric interference does not hinder binding of the molecular recognition element to its biological target. In other preferred embodiments, a crosslinking agent is chosen that does not alter the binding specificity of the molecular recognition element (*See e.g.*, Brinkley, Bioconj. Chem. 3:2-13 [1992]; Zapalinsky, Bioconj. Chem.6:150-65 [1995]; each incorporated herein by reference). In some preferred embodiments, the sequence CDCRGDCFC (*i.e.*, RGD peptide; SEQ ID NO:1) is conjugated using HISTAG or succinimidyl ester to primary amine linkers.

In some preferred embodiments, the fiberless radiative effectors include a co-polymer with a primary amine group (*e.g.*, APMA, acryloamidodextran, aminomethylstyrene, 2-hydroxyethyl acrylate, 2-hydroxymethacrylate, 4-hydroxybutyl acrylate; *See e.g.*, Daubresse *et al.*, J. Pharm. Pharmacol. 45(12):1018-23 [1993]; Noguchi *et al.*, J. Biomed. Mat. Res. 39(4):621-9 [1998]). In some preferred embodiments, the copolymer (*e.g.*, APMA) is itself

partially functionalized and binds the molecular recognition element directly. In other embodiments, the fiberless radiative effectors are crosslinked to molecular recognition elements that contain amine groups via a homobifunctional crosslinking agent that is reactive towards amine groups (*e.g.*, disuccinimidyl suberate, bis(sulfosuccinymidyl)suberate, disuccinimydal glutarate, dimethyladipimidate-2HCl, or dimethylpimelimidate-2HCl). In other embodiments, the amine containing fiberless radiative effectors are cross-linked to proteins containing thiol groups via a heterobifunctional crosslinker that is reactive towards thiols and primary amines (*e.g.*, succinimydyl 3-(2-pyridyldithio)propionate (SPDP); succinimidyl trans-4-(N-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC); succinimidyl (acetylthio)acetate (SATA); 4-[(succinimydyloxy)carboxyl]-α-methyl-α-(2-pyridyldithio)toluene (SMPT); succinimidyl 4-[(iodoacetyl0amino]methyl]-cyclohexane-1-carboxylate (SIAC); m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), or succinimydyl 4-(p-maleimdophenyl)-butyrate (SMPB)). In still other embodiments, the amine containing fiberless radiative effector is crosslinked to a glycoprotein (*i.e.*, carbohydrate containing protein) or carbohydrate via a heterobifunctional crosslinking reagent that is reactive towards both carbohydrates and primary amines or thiols (*e.g.*, 4-[N-Maleimidomethyl]cyclohexane-1-carboxylhydrazide-HCl-1/2 dioxane, 4-[4-Maleimidophenyl]butyric acid hydrazide-HCl). In other embodiments, the peptide molecular recognition elements are directly cross-linked to the cloaking material by choosing an appropriate bifunctional cross-linking agent as described above. In other embodiments, the co-polymer is preferably a semitelechelic poly[N-(2-hydroxypropyl)methacrylamide] with a functional end group (*e.g.*, containing a carboxyl, methyl ester, hydrazide, or amino group) (*See. e.g.*, Lu *et al.*, Bioconj. Chem. 9(6):793-804 [1998], incorporated herein by reference). In still other embodiments, the co-polymer is preferably a poly(amidoamino) formed by addition of amines to bisacrylamide (*See e.g.*, Ferruti *et al.*, Biomaterials 15(15):1235-41 [1994], incorporated herein by reference). In still other embodiments, the co-polymer is preferably polypropylene functionalized with primary amino groups (*See e.g.*, Das and Lindstrom, Biochem. 30(9):2470-7 [1991]; Geysen, Immunol. Meth. 102:259-74 91987], both incorporated herein by reference).

In some embodiments, functionalized derivatives of poly(ethylene glycol) allow convenient attachment of molecular recognition elements to the fiberless radiative effector (*See e.g.*, Zalipsky, Bioconj. Chem. 495):314-18 [1993], incorporated herein by reference). In some preferred embodiments, the functionalized poly(ethylene glycol) is used as either a co-polymer or as a cloaking material for the fiberless radiative effector. In some embodiments, liposomes or fiberless radiative effectors are prepared (*i.e.*, cloaked) from monomethoxypoly(ethylene glycol), to which oligonucleotides and peptides are conjugated (*See e.g.*, Bonora *et al.*, Farmaco 53:634-7 [1998]; Cheng *et al.*, Cancer Immunol. Immunother. 44(6):305-15 [1997]). In other embodiments, peptide molecular recognition elements may be conjugated to polyethyleneglycol acetaldehyde by reductive amination (*See e.g.*, Bentley *et al.*, J. Pharm. Sci. 87(11):1446-9 [1998]). In other embodiments, the cloaking material is preferably a N-hydroxysuccinimide ester of polyethylene glycol to which molecular recognitions elements containing primary amine groups are attached (*See e.g.*, Clark *et al.*, J. Biol. Chem. 271(36)21969-77 [1996], incorporated herein by reference). In still other embodiments, the amino terminal serine or threonine of some peptides is modified on the N-terminal by periodate oxidation to form reactive carbonyl group (*e.g.*, glyoxylyl group). The N-terminal carbonyl group is then reacted with aminooxy-functionalized poly(ethylene glycol) (Gaertner and Offord, Bioconj. Chem. 7(1):38-44 [1996], incorporated herein by reference). In some embodiments, the peptides not normally having a N-terminal serine or threonine are modified to contain one or the other. In still other embodiments, a thiol-protected poly(ethylene glycol) intermediary that reacts with cysteine residues in peptides is preferably produced from monomethoxy poly(ethylene glycol)(Woghiren *et al.*, Bioconj. Chem. 4(5):314-18 [1993], incorporated herein by reference). In some embodiments, liposomes or fiberless radiative effectors are prepared (*i.e.*, cloaked) with a dipalmitoylphosphatidylethanolamine derivative of PEG with a terminal maleimidyl group to which thiol containing compounds (*e.g.*, antibodies) are conjugated (*See e.g.*, Maruyama *et al.*, FEBS Letters 413:177-180 [1997], incorporated herein by reference).

In still further embodiments, the fiberless radiative effector comprises a polymer matrix containing carbohydrate, sulfhydryl, or some other functionalizable group. An appropriate

heterobifunctional or homobifunctional crosslinking reagent is selected as described above and used to covalently attach the fiberless radiative effector to the peptide molecular recognition element. In still further embodiments, molecular recognition elements containing carbohydrate groups by crosslinked directly to the fiberless radiative effector matrix or cloaking material as described.

In some embodiments, two or more molecular recognition elements are attached to the fiberless radiative effector or cloaking materials. For example, in some embodiments, the fiberless radiative effector is biotinylated and crosslinked to another molecular recognition element (*e.g.*, RGD peptide, IL13, or an antigen binding protein) as described above. In other embodiments, the fiberless radiative effector is crosslinked or attached to avidin and some other peptide or protein.

Attachment of more than two molecular recognition elements to a fiberless radiative effector allows their utilization in various stacking approaches. For example, in some embodiments, a first fiberless radiative effector includes a first molecular recognition element (*e.g.*, antigen binding protein, RGD peptide, IL13, or sialic acid), a second molecular recognition element *(e.g.*, biotin), and a radiodynamic compound. A second fiberless radiative effector includes a gamma ray emitter and is crosslinked to avidin. The first fiberless radiative effector binds to a biological target (*i.e.*, pathogen or tumor cell) via the first molecular recognition element, and the second fiberless radiative effector binds to the first fiberless radiative effector via avidin-biotin binding to form a biological target-first fiberless radiative effector-biotin-avidin-second fiberless radiative effector complex. The gamma-rays emitted from the second fiberless radiative effector excite the radiodynamic compound resulting in the production of singlet oxygen and free radicals. As another example, in some embodiments, a first fiberless radiative effector includes a first molecular recognition element (*e.g.*, antigen binding protein, RGD peptide, IL13, or sialic acid), a second molecular recognition element *(e.g.*, biotin), and contains a photodynamic compound. A second fiberless radiative effector includes an enzyme that catalyzes a bioluminescent reaction (*e.g.*, bacterial or insect luciferase) and is crosslinked to avidin. The first fiberless radiative effector binds to a biological target (*i.e.*, pathogen or tumor cell) via the first molecular recognition element,

and the second fiberless radiative effector binds to the first fiberless radiative effector via avidin-biotin binding. Photons produced by the action of the enzyme on a substrate (*e.g.*, luciferin) excite the photodynamic compound in the first fiberless radiative effector so that singlet oxygen and free radicals are produced.

C. Linkage of Other Toxic Agents to Fiberless Radiative Effectors

The present invention also contemplates that a variety of additional toxic agents may be conjugated to or incorporated into the fiberless radiative effector, cloaking material, or liposome as described above. Such toxic agents may be utilized in fiberless radiative effectors in conjunction with, for example, photodynamic compounds or radiodynamic compounds, or by themselves as the primary toxic agent. Examples of such toxic agents and the necessary linkage are given in Table 1.

Table 1

Toxic Agents		
Name	**Linkage**	**Source**
Antimicrobials		
Streptomycin	ester/amide	
Neomycin	ester/amide	Dow, Lilly
Amikacin	ester	Bristol
Gentamicin	ester/amide	Upjohn
Tobramycin	ester/amide	Lilly
Streptomycin B	ester/amide	Squibb
Spectinomycin	ester	Upjohn
Ampicillin	amide	Squibb

Table 1

Sulfaniliamide	amide	Merrell-National
Polymixin	amide	Burroughs-Wellcome
Chloramphenicol	ester	Parke-Davis
Antivirals		
Acyclovir		Burroughs-Wellcome
Vira A	ester/amide	Parke-Davis
Symmetrel	amide	Endo
Antifungals		
Nystatin	ester	Squibb
Antineoplastics		
Adriamycin	ester/amide	Adria
Cerubidine	ester/amide	Ives
Bleomycin	ester/amide	Bristol
Alkeran	amide	Burroughs-Wellcome
Velban	ester	Lilly
Oncovin	ester	Lilly
Fluorouracil	ester	Adria
Methotrexate	amide	Lederic
Thiotepa		Lederic
Bisantrene		Lederic

Table 1

Novantrone	ester	Lederic
Thioguanine	amide	Burroughs-Wellcome
Procarabizine		Hoffman-La Roche
Cytarabine		Upjohn
Heavy Metals		
Barium		
Gold		
Platinum		
Antimycoplasmals		
Tylosine		
Spectinomycin		

VIII. Uses of Fiberless Radiative Effectors

The fiberless radiative effectors of the present invention are utilized in a variety of *in vivo* and *in vitro* procedures. In some embodiments, the fiberless radiative effectors are utilized to destroy or inhibit the growth of a biological target (*e.g.*, pathogens, macromolecules, or tumor cells in culture or in the body). In other embodiments, the fiberless radiative effectors are used as markers (*e.g.*, fluorescent markers or magnetic markers) for the detection of a biological target.

A. Use for Photodynamic and Radiodynamic Therapy of Tumors

In some embodiments of the present invention, fiberless radiative effectors comprising molecular recognition elements that specifically bind to tumor cells (*e.g.*, gliomas or

squamous cancer cells) are used to treat patients with cancer. A variety of molecular recognition elements are attached to such fiberless radiative effectors. In some embodiments, the molecular recognition element is a peptide or protein (*e.g.*, RGD peptide or IL13) that acts as a ligand for a tumor cell specific protein (*e.g.*, cell membrane receptor, extracellular matrix protein). In other embodiments, the molecular recognition elements is an antigen binding protein (*e.g.*, antibody, single chain antibody, or Fab fragment) that binds a tumor cell specific antigen. Examples of such antigen binding proteins and antigens are described above.

1. Malignant Glioma

Among the causes of death due to cancer, brain tumors are ranked second in the pediatric age group and fourth in middle-aged men. Despite the use of multimodality therapy, malignant gliomas are uniformly fatal; only 50% of patients survive even one year from the time of diagnosis. These stark statistics underscore the urgent need for novel therapeutic strategies and delivery vehicles for the treatment of these tumors.

Primary intracranial neoplasms occur at a rate of 4.5-8.2 per 100,000 people per year. In the United States, an estimated 9,000 to 17,000 new primary tumors are diagnosed each year. Despite recent advances in neurosurgery, neuroradiology, and neurooncology, the multimodality approach currently used in the treatment of malignant brain tumors produces few cures. Malignant gliomas constitute a major clinical problem because of their frequency of occurrence and extremely poor prognosis. Although neurosurgical resection can remove 90% of a malignant astrocytoma, a residual tumor may still contain 10^{10} cells. Radiation and chemotherapy are typically given following surgical resection yet the median life span remains less than 1 year with fewer than 20% of patients surviving 2 years from initial diagnosis. Immunotherapy has also proven to be inadequate due to the relatively large numbers of glioma cells surviving initial immunological responses. Although many technical obstacles have been overcome, the most recent strategies, including the use of hyperthermia in the treatment of these tumors, have proven unfruitful in extending the life expectancy of patients with malignant gliomas. Although there are numerous reasons for the lack of significant

progress in the treatment of many cranial tumors, the unfavorable results obtained to date in the treatment of malignant astrocytomas underscores the need for developing and investigating novel and aggressive therapeutic approaches to treat these diseases.

2. Head and Neck Cancer

Head and neck cancer is any type of cancer of the upper airways/digestive tract, face, or neck. In 1995, about 50,000 head and neck cancers were expected to be diagnosed in this country. Over 13,000 people were expected to die from the condition. Head and neck cancers are three times more common in men than in women. Over 90 percent of head and neck cancers are squamous cell carcinomas. Excessive use of alcohol or tobacco is the most common risk factor for the disease and is associated with over 95 percent of cases. Use of both alcohol and tobacco (including smokeless tobacco) significantly increases risk for the condition. Exposure to toxic fumes and chemicals may predispose an individual to cancers of the oral cavity and throat.

Two-thirds of all patients with squamous cell carcinoma of the head and neck (SCCHN) present with locally advanced disease. For these patients, the treatment offering the best chance of local control/cure consists of surgery (which is often disfiguring and functionally morbid) followed by postoperative radiotherapy (RT). For patients with medically inoperable or unresectable locally advanced disease, radiotherapy alone is the standard treatment; however, results of such treatment are unsatisfying with 5 year survival rates of less than 25% and most treatment failures occurring locally or regionally (*i.e.*, within the irradiated fields). Since only 10% of patients with SCCHN develop metastatic disease, it is clear that the development of improved non-surgical treatments for locally advanced disease would represent a significant advance in the treatment of unresectable/inoperable locally advanced SCCHN and might provide less morbid, organ-preserving options for patients with resectable disease. The combination of chemotherapy and RT has been extensively studied as a strategy to improve the results of non-surgical therapy for SCCHN. Unfortunately, despite promising responses to the concurrent administration of chemotherapy and radiation, the clinical utility

of concurrent chemotherapy and radiation as the initial definitive non-surgical management of locally advanced SCCHN remains limited by the increased toxicity (mucosal as well as systemic) of the combined treatments.

3. Treatment of Malignant Glioma and Head and Neck Cancer

In some preferred embodiments, fiberless radiative effectors with IL13 molecular recognition elements are used to treat patients who have malignant gliomas. In some embodiments, the fiberless radiative effector for treatment of glioma comprises a polymer matrix (*e.g.*, acrylamide or decylacrylate) including a toxic agent, preferably a photodynamic compound (*e.g.*, ruthenium red or Photofrin II), a cloaking material (*e.g.*, PEG or a liposome) and IL13 attached to either the cloaking material or the polymer matrix (*e.g.*, via a heterobifunctional or homobifunctional crosslinker or biotin as described above). In some embodiments, the fiberless radiative effectors administered to the patient intravenously. The fiberless radiative effectors are then allowed to bind to the tumor cells. In some embodiments, the fiberless radiative effectors are illuminated (*e.g.*, by fiber optics or a laser, or combination thereof) so that the photodynamic compound is excited, resulting in the production of singlet oxygen and free radicals (*e.g.*, superoxide or hydroxyl radicals). In some preferred embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector into the tumor cells, resulting in tumor necrosis. In other preferred embodiments, head and neck cancer is treated as described, except that the RGD peptide is utilized as the molecular recognition element.

In other preferred embodiments, the fiberless radiative effector for treatment of glioma comprises a polymer matrix (*e.g.*, poly(styrene)) including a toxic agent, preferably a radiodynamic compound (*e.g.*, 2,5-diphenyloxazole (PPO)), a photodynamic compound (*e.g.*, Photofrin II), a cloaking material (*e.g.*, PEG or a liposome) and IL13 attached to either the cloaking material or the polymer matrix (*e.g.*, via a heterobifunctional or homobifunctional crosslinker or biotin). In some embodiments, the fiberless radiative effectors administered to the patient intravenously. The fiberless radiative effectors are then allowed to bind to the

tumor cells. In some embodiments, the fiberless radiative effectors are exposed to X-rays so that energy (*i.e.*, excitons) is transferred to the photodynamic compound, resulting in the production of singlet oxygen and free radicals (*e.g.*, superoxide or hydroxyl radicals). In some preferred embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector into the tumor cells, resulting in tumor necrosis. In other preferred embodiments, head and neck cancer is treated as described, except that the RGD peptide is utilized as the molecular recognition element.

In other preferred embodiments, the fiberless radiative effector for treatment of glioma comprises a polymer matrix (*e.g.*, poly(styrene)) including a toxic agent, preferably a radiodynamic compound (*e.g.*, 2,5-diphenyloxazole (PPO)), a photodynamic compound (*e.g.*, Photofrin II), a cloaking material (*e.g.*, PEG or a liposome); IL13 attached to either the cloaking material or the polymer matrix (*e.g.*, via a heterobifunctional or homobifunctional crosslinker or biotin), and biotin attached to either the cloaking material or polymer matrix. In some embodiments, the fiberless radiative effectors administered to the patient intravenously. The fiberless radiative effectors are then allowed to bind to the tumor cells. In some embodiments, a second fiberless radiative effector is provided that comprises a polymer matrix including a gamma ray emitter, cloaking material, and avidin attached to either the polymer matrix or the cloaking material. In some embodiments, the second fiberless radiative effectors are administered intravenously so that they bind to the first fiberless radiative effectors. The first fiberless radiative effectors are exposed to gamma-rays so that energy (*i.e.*, excitons) is transferred from the radiodynamic compound to the photodynamic compound, resulting in the production of singlet oxygen and free radicals (*e.g.*, superoxide or hydroxyl radicals). In some preferred embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector into the tumor cells, resulting in tumor necrosis. In other preferred embodiments, head and neck cancer is treated as described, except that the RGD peptide is utilized as the molecular recognition element.

B. Use in Photodynamic Therapy of Pathogen Exposure

In some embodiments of the present invention, fiberless radiative effectors comprising molecular recognition elements that specifically bind to pathogens (*e.g.*, *Legionella peomophilia*, *Mycobacterium tuberculosis*, *Clostridium tetani*, *Hemophilus influenzae*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Bacillus anthracis*, *Vibrio cholerae*, *Borrelia burgdorferi*, *Cornebacterium diphtheria*, *Staphylococcus aureus*, human papilloma virus, human immunodeficiency virus, rubella virus, polio virus, and the like) are used to treat patients who have been exposed to the pathogen. In some embodiments, fiberless radiative effectors comprise a polymeric matrix, a photodynamic compound, a cloaking agent, and a molecular recognition element that binds to a pathogen epitope (described in detailed above). In some embodiments, a patient who has been exposed to a pathogen is treated by intravenous administration of a fiberless radiative effector that recognizes the pathogen. In some embodiments, about 10^8 to 10^{18} (each about 50 nm), most preferably about 10^{14} fiberless radiative effectors, are administered (*e.g.*, orally or intravenously) to the patient. In some embodiments, each fiberless radiative effector contains about 0.1 to 10% of the photodynamic compound, preferably about 1.0% of the photodynamic compound. In other embodiments, about 1 μg/kg to 1000 μg/kg, most preferably about 100 μg/kg, fiberless radiative effectors are administered. In some embodiments, the fiberless radiative effectors are then allowed a period of time to bind the pathogen, preferably about 1 minute to 24 hours, most preferably for about an hour, resulting in the formation of a pathogen-fiberless radiative effector complex. In some embodiments, the fiberless radiative effectors are then illuminated (*e.g.*, with a red laser, incandescent lamp, or filtered sunlight). In some embodiments, the light is aimed at the jugular vein or some other superficial blood or lymphatic vessel. In some embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector to the pathogen, causing its destruction.

In other embodiments, fiberless radiative effectors comprise a polymeric matrix, a radiodynamic compound, a photodynamic compound, a cloaking agent, and a molecular recognition element that binds to a pathogen epitope (described in detailed above). In some

embodiments, a patient who has been exposed to a pathogen is treated by intravenous administration of a fiberless radiative effector that recognizes the pathogen. In some preferred embodiments, about 10^8 to 10^{18} (each about 50 nm), most preferably about 10^{14} fiberless radiative effectors, are administered to the patient. In some embodiments, each fiberless radiative effector contains about 0.1 to 10% of the photodynamic compound, preferably about 1.0% of the photodynamic compound. In other embodiments, about 1 μg/kg to 1000 μg/kg, most preferably about 100 μg/kg, fiberless radiative effectors are administered. In some embodiments, the fiberless radiative effectors are then allowed a period of time to bind the pathogen, preferably about 1 minute to 24 hours, most preferably for about an hour, resulting in the formation of a pathogen-fiberless radiative effector complex. In some embodiments, the fiberless radiative effectors are then exposed to X-rays, resulting in the production of singlet oxygen and free radicals as described above. In some embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector to the pathogen, causing its destruction.

In still other embodiments, fiberless radiative effectors comprise a polymeric matrix, a radiodynamic compound, a photodynamic compound, a cloaking agent, biotin and a molecular recognition element that binds to a pathogen epitope (described in detailed above). In some embodiments, a patient who has been exposed to a pathogen is treated by intravenous administration of a fiberless radiative effector that recognizes the pathogen. In some preferred embodiments, about 10^8 to 10^{18} (each about 50 nm), most preferably about 10^{14} fiberless radiative effectors, are administered to the patient. In some embodiments, each fiberless radiative effector contains about 0.1 to 10% of the photodynamic compound, preferably about 1.0% of the photodynamic compound. In other embodiments, about 1 μg/kg to 1000 μg/kg, most preferably about 100 μg/kg, fiberless radiative effectors are administered. In some embodiments, the fiberless radiative effectors are then allowed a period of time to bind the pathogen, preferably about 1 minute to 24 hours, most preferably for about an hour, resulting in the formation of a pathogen-fiberless radiative effector complex. In some embodiments, a second fiberless radiative effector is provided comprising a polymeric matrix, a gamma ray

emitter, a cloaking agent, and avidin. The second fiberless radiative effector is administered intravenously at similar concentration as the first fiberless radiative effector. In some embodiments, the second fiberless radiative effector binds to the first fiberless radiative effector, thereby exposing the first fiberless radiative effector radiodynamic compound to gamma rays. In some embodiments, energy (*i.e.*, excitons) is transferred from the radiodynamic compound to the photodynamic compound, causing the excitation of the photodynamic compound and production of singlet oxygen and radicals. In some embodiments, the singlet oxygen and free radicals diffuse from the fiberless radiative effector to the pathogen, causing its destruction.

C. Use of Fiberless Radiative Effectors for Imaging

In some embodiments of the present invention, the fiberless radiative effectors are used for imaging or detecting a biological target. In some preferred embodiments, a signaling material (e.g., magnetic particle, fluorescent compound, or bioluminescent compound) or other detectable material is incorporated into the fiberless radiative effector polymeric matrix. It is desirable that the material is easily detectable at low levels so that the imaging is conducted *in situ*. In this manner, the response of a biological target (*e.g.*, tumors) to treatment can be assayed. A major advantage of *in situ*, non-invasive measures of tumor response is that a single test subject (*e.g.*, mouse or other animal) can be followed with time. Therefore, tumor volumes can be measured without sacrificing the animal, resulting in the use of fewer animals. *In situ* imaging is also be used to follow tumor regression following treatment in humans. In some embodiments, the detectable material is selected from magnetic materials (*e.g.*, iron for MRI); proteins that catalyze luminescent reactions (*e.g.*, luciferase for bioluminescent imaging); fluorescent dyes (*e.g.*, rodamine or fluorescein isothiocyanate for fluorescent imaging); fluorescent proteins (*e.g.*, green fluorescent protein); and radioactive elements (*e.g.*, for autoradiography).

1. Bioluminescent Imaging

In some embodiments, fiberless radiative effectors of the present invention are used to detect biological targets *in vivo* or *in vitro* by bioluminescent imaging. In some embodiments, fiberless radiative effectors comprise a polymeric matrix (*e.g.*, sol-gel or acrlyamide), an enzyme that catalyzes a bioluminescent reaction, a cloaking agent, and a molecular recognition element (*e.g.*, antigen binding protein, RGD peptide or IL13) that binds to a biological target (*e.g.*, tumor cells, described in detailed above). In some preferred embodiments, the matrix material allows the diffusion of substrates (*e.g.*, luciferin and ATP) to the enzyme. Enzymes that catalyze bioluminescent reactions include, but are not limited to, the following luciferases: bacterial luciferase (U.S. Pat. No. 4,548,994), *Photinus pyralis* luciferase (U.S. Pat. Nos. 5,670,356 and 5,674,713), *Renilla reniformus* luciferase, *Pyrophorus plagiophthalamus* luciferase, *Luciola cruciata* luciferase (Masuda *et al.*, Gene 77:265-70 [1989]), *Luciola lateralis* luciferase (Tatsumi *et al.*, Biochim. Biophys Acta 1131:161-65 [1992]), and *Latia neritoides* luciferase. The foregoing publications are specifically incorporated herein by reference.

In some embodiments, the imaging is performed *in situ* (*See e.g.*, Contag *et al.*, Nature Med. 4(2):245-47 [1998], incorporated herein by reference). Fiberless radiative effectors containing the bioluminescent enzyme are provided to the animal intravenously and allowed time so that the molecular recognition element binds to its biological target. In some embodiments, a substrate (*e.g.*, bacterial or insect luciferin) for the bioluminescent enzyme is then provided (*e.g.*, via intravenous, intraperitoneal, intravesical, or intracerebrovascular delivery) to the animal. In some embodiments, production of bioluminescence by the action of the enzyme on the substrate is then detected by a bioluminescence detection system. In some embodiments, the bioluminescence detection system comprises a Hamamatsu intensified CCD (ICCD, model C2400-32). In other embodiments, the bioluminescence detection system further comprises other devices for intensifying weak signals (*e.g.*, microchannel plate intensifiers and devices for Peltier or liquid nitrogen cooling of the detector and/or intensifier). In some preferred embodiments, a grey scale image of the animal is obtained by

opening the door of dark chamber in which the animal is placed. The door is then shut and the gain on the intensifier adjusted to maximum to detect the bioluminescent signal. The signal is then overlaid with the greyscale image in pseudocolor.

The use of fiberless radiative effectors targeted to a particular biological target has advantages over previously described methods of *in situ* bioluminescent imaging (*See e.g.*, Contag *et al.*, *supra*). The previously described methods require injection of tumor cells that express luciferase into mice. Obviously, this approach can not be transferred to the clinical environment. In contrast, fiberless radiative effectors are non-toxic, can be designed to image virtually any biological target, and can be used in clinical setting.

2. Magnetic Resonance Imaging

In other preferred embodiments, fiberless radiative effectors of the present invention are used to detect biological targets by magnetic resonance imaging (MRI). In some embodiments, fiberless radiative effectors comprise a polymeric matrix (*e.g.*, sol-gel or acrlyamide), a magnetic material (*e.g.*, iron oxide), a cloaking agent, and a molecular recognition element (*e.g.*, antigen binding protein, RGD peptide or IL13) that binds to a biological target (*e.g.*, tumor cells, described in detailed above). In some embodiments, the fiberless radiative effector also comprises a toxic agent, so that the fiberless radiative effector is utilized to both destroy or inhibit the growth of a biological target and image the biological target. Accordingly, the fiberless radiative effector can be used to both monitor tumor growth and response to therapy.

In some embodiments, the biological target imaged is *in situ* (*See e.g.*, Ross *et al.*, PNAS 95:7012-17 [1998]). Fiberless radiative effectors containing the magnetic material are provided to the animal (*e.g.*, intravenously) and time allowed so that the molecular recognition element binds to its biological target. In some embodiments, the biological target is then imaged with a magnetic resonance system (*e.g.*, a 7-Tesla Magnetic Resonance System). In some embodiments, T1-weighted or T2-weighted images are obtained.

In some embodiments, it is contemplated that inclusion of the magnetic material in the fiberless radiative effector enhances photodynamic therapy. It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, it has been shown recently that the photohemolysis of human erythrocytes with the non-steroidal anti-inflammatory agent ketoprofen is dramatically amplified by either a static external magnetic field (Chignell and Sik, Photochem. Photobiol. 67:591-95 [1998]; Chignell and Sik, Photochem. Photobiol. 62:205-7 [1995]) or the fields due to the presence of magnetic nano-particles (Chignell and Sik, Photochem. Photobiol. 68:598-601 [1998]; Scaiano *et al.*, Photochem. Photobiol. 65:759-62 [1997]). The hypothesized mechanism (Scaiano *et al.*, Photochem. Photobiol. 59:585-89 [1994]) involves the formation of triplet radical pairs in the ketoprofen reaction with cell lipids, which often undergo an intersystem crossing to a singlet pair that can react with itself, simply replenishing the initial reactants. In the presence of the magnetic fields, though, the triplet/singlet degeneracy is broken in the radical pair, causing a slowdown in the intersystem crossing rate to self-annihilating singlet pairs, and a concomitant increase in the concentration and/or lifetime of free radicals that escape from the pair, and are thus available for further reactions causing cell damage.

D. Use for Removing Sub-population of Cells from Mixture

In some embodiments of the present invention, the fiberless radiative effectors are used to eliminate a subpopulation of cells from a mixture of cells, thereby creating a purified cell population from the remaining cells. In some embodiments, T-cells may be removed from a sample containing a mixture of T-cells and B-cells. In some preferred embodiments, there is provided a sample containing a mixture of B-cells and T-cells and fiberless radiative effectors which recognize or are specific for T-cells. In some embodiments, the fiberless radiative effectors comprise a toxic agent and molecular recognition element which specifically recognizes T-cells. In other embodiments, the fiberless radiative effectors and cell sample are combined so that the fiberless radiative effectors bind to T-cells contained in the sample. In

still further embodiments, the fiberless radiative effectors are illuminated so that free-radicals are produced, resulting in the depletion of T-cells from the sample.

The present invention is not limited to any one molecular recognition element. Indeed, a variety of molecular recognition elements are contemplated. In some embodiments, the molecular recognition element that specifically recognizes T-cells is the monoclonal antibody OKT3 (ATCC CRL 8001; *See, e.g.*, U.S. Pat. No. 4,731,244). In other embodiments, the molecular recognition element comprises an anti-TAC antibody (*See, e.g.*, Kupiec-Weglinski et al., PNAS 83:2624 [1986]) or another antibody that recognizes T-cells (*See, e.g.*, Abdi *et al.*, J. Immunol. 142(9):2971-80 [1989] and Wang *et al.*, Eur. J. Immunol. 24(7):1549-52 [1994]).

IX. Other Preparations for *In Vivo* Use

The present invention contemplates using therapeutic compositions of fiberless radiative effectors, in particular for treatment of cancer and exposure to pathogens. It is not intended that the present invention be limited by the particular nature of the therapeutic preparation. For example, such compositions can be provided together with physiologically tolerable liquid, gel or solid carriers, diluents, adjuvants and excipients. In addition, the fiberless radiative effectors are used with other therapies, including chemotherapy and radiation therapy for the treatment of cancer, and antibiotic and vaccine therapy for treatment for exposure to pathogens.

With respect to the mode of administration, methods for administration of the fiberless radiative effectors include, but are not limited to, intravenous, intramuscular, intrathecal or topical (including topical ophthalmic) administration. Formulations for such administrations comprise an effective amount of fiberless radiative effectors analog in sterile water or physiological saline.

On the other hand, some formulations contain such normally employed additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium

saccharin, cellulose, magnesium carbonate, and the like. These compositions typically contain 1%-95% of active ingredient, preferably 2%-70%.

The compositions are preferably prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared.

The fiberless radiative effectors of the present invention are often mixed with diluents or excipients that are compatible and physiologically tolerable. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

Likewise, dosage ranges for fiberless radiative effector treatment include, but are not limited to: 1 ng/kg/day to 200 mg/kg/day.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be read as limiting the scope thereof.

Example 1

Synthesis of PEG-Cloaked Acrlyamide Fiberless Radiative Effector Containing Ruthenium Dye and Cross-Linked to RGD Peptide

This example describes the synthesis of fiberless radiative effectors comprising an acrylamide core, a RGD peptide molecular recognition element, a photodynamic compound, and PEG cloaking material.

Preparation of Acrylamide Nanospheres: The polymerization solution was made by combining 50 mg ruthenium dye, 27% acrylamide, 3% N,N-methylenebis(acrylamide), in 1 mL of 10 mM phosphate buffer, pH 7.4. The polymerization solution was then added to a solution containing 20 mL hexane, 1.8 mmol dioctyl sulfosuccinate sodium salt and 4.24

mmol Brij 30 (4 Lauryl ether), and the two solutions were emulsified by stirring. The polymerization was initiated with 50 μL of a 10% sodium bisulfite solution OR 24 μL of a 10% ammonium persulfate solution and 12 μL N,N,N,N-tetramethylethylenediamine (TEMED), and the solution was stirred at room temperature for 2 hours. Hexane was removed by rotary evaporation and then the effectors were precipitated by the addition of ethanol. Excess surfactant and dye were removed by rinsing with ethanol, to yield a product consisting of 20 and 200 nm probes.

PEG Cloaking: Nanospheress are diluted in deionized water to 0.3 % (w/v), then added to an equal volume of -hydroxy PEG (2 % w/v) (Aldrich, Milwaukee, WI) in water and incubated overnight at room temperature.

RGD crosslinking: Fiberless radiative effectors are produced by stirring the PEG cloaked nanospheres with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope. The biotinylated fiberless radiative effectors are then incubated with avidin linked RGD, and incubated for three hours at room temperature. The final product is rinsed and filtered with phosphate buffer.

Example 2

Synthesis of Decyl-Methacrylate Fiberless Radiative Effector Cross-Linked to IL13

Preparation of Cloaked Decyl-methacrylate Nanospheres: To produce the nanospheres, HCl is placed in a two necked, 100ml round bottom flask and nitrogen is slowly run through the flask while the solution is stirring. A cloaking agent, oxidized dextran (also called side-on dextran) (440 mg) is then added to the flask. The decyl methacrylate (55 mg), hexanedioldimethacrylate (75 mg), Photophrin II (1 mg) and DOS (100 mg) are weighed out in a small scintillation vial. The round bottom flask is capped and transferred to the sonicator. Decyl methacrylate, hexandioldimethacrylate, and DOS are transferred to the round bottom flask using two 1ml aliquots of ethanol. The solution sonicates for 5-10 minutes and then is transferred back to the stir plate where the slow flow of nitrogen is reinstated and a

condenser is added to the flask. The flask is heated to 80°C and allowed to react over night. The solution is suction filtered to obtain the fiberless radiative effectors. After a THF soak and additional filtration the desired dye and ionophores can be added using fresh DOS plasticizer.

Crosslinking of IL13: To produce the fiberless radiative effectors, the dextrate-linked nanospheres are stirred with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope. The biotinylated fiberless radiative effectors are then incubated with avidin-linked IL13, and incubated for three hours at room temperature. The final product is rinsed and filtered with phosphate buffer.

Example 3

Synthesis of Biotinylated Fiberless Radiative Effector

This example describes the synthesis of a biotinylated fiberless radiative effector which can be bound to biotinylated molecular recognition elements via avidin or directly to molecular recognition elements that are directly bound or conjugated to avidin.

Preparation of Acrylamide Nanospheres: The polymerization solution was made by combining 50 mg ruthenium dye, 0.88 g acrylamide, 0.30 g N,N-methylenebis(acrylamide) and 250 µL N-(3-Aminopropyl) methacrylamide hydrochloride (APMA), in 15 mL of 10 mM phosphate buffer, pH 7.4. One mL of polymerization solution was then added to a solution containing 20 mL hexane, 1.8 mmol dioctyl sulfosuccinate sodium salt and 4.24 mmol Brij 30 (4 Lauryl ether), and the two solutions were emulsified by stirring. The polymerization was initiated with 50 µL of a 10% sodium bisulfite solution OR 24 µL of a 10% ammonium persulfate solution and 12 µL N,N,N,N-tetramethylethylenediamine (TEMED), and the solution was stirred at room temperature for 2 hours. Hexane was removed by rotary evaporation and then the effectors were precipitated by the addition of ethanol. Excess surfactant and dye were removed by rinsing with ethanol, to yield a product consisting of 20 and 200 nm probes.

Biotinylation of Acrylamide Nanospheres: Dry nanospheres (27 mg) are suspended in 1000 μL and 3 mg of NHS-biotin is added. The solution is stirred at room temperature for 1 hour, then filtered and rinsed with sodium bicarbonate.

Example 4

Synthesis of Fiberless Radiative Effector Biotinylated on the PEG Cloak

This example described the synthesis of fiberless radiative effectors which are biotinylated on their PEG cloaks. Biotinylated molecular recognition elements can be attached to the to the fiberless radiative effector via an avidin linker, or molecular recognition elements bound or conjugated directly avidin can be attached to the fiberless radiative effector.

Preparation of Acrylamide Nanospheres: The polymerization solution was made by combining 50 mg ruthenium dye, 27% acrylamide, 3% N,N-methylenebis(acrylamide), in 1 mL of 10 mM phosphate buffer, pH 7.4. The polymerization solution was then added to a solution containing 20 mL hexane, 1.8 mmol dioctyl sulfosuccinate sodium salt and 4.24 mmol Brij 30 (4 Lauryl ether), and the two solutions were emulsified by stirring. The polymerization was initiated with 50 μL of a 10% sodium bisulfite solution OR 24 μL of a 10% ammonium persulfate solution and 12 μL N,N,N,N-tetramethylethylenediamine (TEMED), and the solution was stirred at room temperature for 2 hours. Hexane was removed by rotary evaporation and then the effectors were precipitated by the addition of ethanol. Excess surfactant and dye were removed by rinsing with ethanol, to yield a product consisting of 20 and 200 nm probes.

PEG Cloaking: Nanospheres are diluted in deionized water to 0.3 % (w/v), then added to an equal volume of -hydroxy PEG (2 % w/v) in water and incubated overnight at room temperature.

Biotin Linkage: The PEG cloaked nanospheres are stirred with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope.

Example 5

Synthesis of Fiberless Radiative Effector Containing a Co-Polymer with Primary Amine Groups

This example describes the synthesis of a fiberless radiative effector containing a co-polymer with primary amine groups. The primary amine groups serve as convenient points of attachment for a variety of cross-linking agents.

Preparation of Acrylamide Nanospheres: The polymerization solution was made by combining 50 mg ruthenium dye, 0.88 g acrylamide, 0.30 g N,N-methylenebis(acrylamide) and 250 μL N-(3-Aminopropyl) methacrylamide hydrochloride (APMA), in 15 mL of 10 mM phosphate buffer, pH 7.4. One mL of polymerization solution was then added to a solution containing 20 mL hexane, 1.8 mmol dictyl sulfosuccinate sodium salt and 4.24 mmol Brij 30 (4 Lauryl ether), and the two solutions were emulsified by stirring. The polymerization was initiated with 50 μL of a 10% sodium bisulfite solution or 24 μL of a 10% ammonium persulfate solution and 12 μL N,N,N,N-tetramethylethylenediamine (TEMED), and the solution was stirred at room temperature for 2 hours. Hexane was removed by rotary evaporation and then the effectors were precipitated by the addition of ethanol. Excess surfactant and dye were removed by rinsing with ethanol, to yield a product consisting of 20 and 200 nm probes.

Linkage of IL13 (or alternate peptide) to Acrylamide Nanospheres: Dry nanospheres (27 mg) are suspended in 1000 μL and 5 mg of IL13 is added. The solution is stirred at room temperature for 1 hour, then filtered and rinsed with sodium bicarbonate. Note that a cross-linking agent is not required because of the intrinsic reactivity of the APMA. However, a homobifunctional or heterobifunctional cross-linking agent could be used if desired.

Example 6

Synthesis of Sol-Gel Fiberless Radiative Effector Attached to a Antigen Binding Protein

This example describes the synthesis of a PEG-cloaked sol-gel fiberless radiative effector with an antigen binding protein molecular recognition element (*i.e.*, TAG72).

Preparation of Sol-Gel Nanospheres: A microemulsion solution consisting of 20 mL cyclohexane, 120 μL NH_3H_2O (29% wt.), 100 μL Tetraethylorthosilicate (TEOS), 2.3g polyoxyethylene (4) nonphenyl ether (NP-4) and 50 μg Ruthenium dye was stirred overnight at room temperature. The cyclohexane was removed by rotary evaporation and the fiberless radiative effectors were precipitated by the addition of ethanol and collected by filtration.

PEG Cloaking: The nanospheres were silanized by suspending them in a solution containing 2% aqueous 3-(Trimethoxysilyl)propylmethacrylate adjusted to pH 3.45 and stirring for 2 hours at room temperature. The silanized fiberless radiative effectors were then dialyzed with phosphate buffer and diluted in deionized water to 0.3 % (w/v), then added to an equal volume of -hydroxy PEG (2 % w/v) in water and incubated overnight at room temperature.

TAG72 Antibody Crosslinking: The PEG linked sol-gel nanospheres are stirred with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope. The biotinylated fiberless radiative effectors are then incubated with avidin linked TAG72, and incubated for three hours at room temperature. The final product is rinsed and filtered with phosphate buffer.

Example 7

Synthesis of PEG-Cloaked PVA Fiberless Radiative Effectors

This example describes the synthesis of fiberless radiative effectors from poly(vinyl alcohol).

Preparation of PEG cloaked PVA Nanospheres: PVA (500 mg) was dissolved in 3 mL phosphate buffer (20 mM, pH 7.4) by heating at 90°C. When the PVA solution had cooled to room temperature it was added to 2 mL phosphate buffer which contained 50 mg ruthenium dye and methoxypoly(ethylene glycol) acrylate (2% w/v) and stirred vigorously. This aqueous solution was added to 100 ml silicone oil and the mixture was homogenized for 5 min to form a water-in-oil emulsion. The w/o emulsion was then frozen at -20°C for 20 hours and

then allowed to thaw for 4 hours. Three such freezing-thawing cycles were performed to produce a suspension of PVA-PEG nanoparticles in silicone oil. The PVA-PEG nanoparticles were extracted from the silicone oil using acetone and collected by vacuum filtration. The nanoparticles were washed with acetone to remove any residual silicone oil and stored at 4°C.

Cross-linkage to a Peptide: Dry PVA-PEG Fiberless Radiative Effectors (27 mg) are suspended in 1000 μL and 5 mg of IL13 (peptide) is added. The solution is stirred at room temperature for 1 hour, then filtered and rinsed with sodium bicarbonate.

Example 8

Synthesis of Radiodynamic Fiberless Radiative Effectors

This example describes the synthesis of radiodynamic fiberless radiative effectors. Polystyrene nanoparticles containing photodynamic compound *(e.g.*, Ruthenium dye) can be purchased commercially (Bangs Laboratories, Fisher, IN).

PEG Cloaking: The polystyrene nanospheres were reacted with the macromonomer methoxypoly(ethylene glycol) acrylate in the molar ratio 1:0.025 using potassium persulfate as the initiator. The polystyrene-PEG (PS-PEG) particles were then filtered and dialyzed.

Radiodynamic Labelling: The PS-PEG particles were surface labelled with NaI-125 (Amersham Int'l. U.K., an iodine radiolabel). NaI-125 (2.5 μL, 3.7 GBq/ml) was mixed with 1 mL of PS-PEG particle dispersion (2% w/v) and exposed to a Cesium-137 source for 48 hours. After irradiation, excess free iodine was removed by dialysis.

Crosslinking of Peptide and Avidin: The radiolabelled PS-PEG nanospheres are stirred with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope. The biotinylated fiberless radiative effectors are then incubated with avidin linked IL13 (or some other peptide), and incubated for three hours at room temperature. The final product is rinsed and filtered with phosphate buffer.

Example 9

Synthesis of Fiberless Radiative Effector Containing a Gamma Ray Producer

This example describes the synthesis of a fiberless radiative effector containing a gamma ray producer. This fiberless radiative effector may be used in conjunction with the radiodynamic fiberless radiative effector described in Example 8 or by itself.

Preparation of Acrylamide Nanospheres: Fiberless radiative effectors are produced using the methods described in Example 1, except that Ruthenium dye in the polymerization solution is replaced with a gamma ray producer (^{111}In-oxine, 37 MBq/ml, Amersham Int'l) and ascorbic acid (35 mg) as an antioxidant.

PEG Cloaking: Nanospheres are diluted in deionized water to 0.3 % (w/v), then added to an equal volume of -hydroxy PEG (2 % w/v) in water and incubated overnight at room temperature.

Biotin linkage: The PEG cloaked nanospheres are stirred with NHS-biotin and triethylamine in dichloromethane and acetonitrile at room temperature under nitrogen overnight. The fiberless radiative effectors are then isolated by vacuum filtration and dried from toluene azeotrope.

Example 10

Synthesis of Fiberless Radiative Effector Containing Magnetic Material

This example describes the synthesis of fiberless radiative effectors containing magnetic material. These fiberless radiative effectors may be imaged by magnetic resonance and can be used to both destroy a biological target and monitor the effect of the therapy. Briefly, fiberless radiative effectors are prepared as in Examples 1 or 2, but with the addition of monocrystalline iron oxide nanoparticles (MION) at the polymerization solution stage.

Example 11

Synthesis of Fiberless Radiative Effectors Contained in Liposome Cloak

This example describes the synthesis of fiberless radiative effectors comprising a cloak. The liposome material includes a PEG derivative which can be biotinylated so that molecular recognition elements can be attached to the liposome for biological targeting.

PEG-linked Liposomes Containing Fiberless Radiative Effectors Preparation: A mixture of phosphatidylcholine (PC) and cholesterol (1:1 molar ratio) was mixed with 7.4 mol% of PEG-PE (PEG linked dioleoylphosphatidylethanolamine) in dichloromethane/ethanol in a round bottom flask. The organic solvent was evaporated under nitrogen, then vacuum desiccated for 2 hrs. Normal saline containing a suspension of nanospheres (5 %) was added and the lipid suspension was vortexed for 2 min and extruded ten times through two stacked Nucleopore polycarbonate filters (400 nm pore size).

Crosslinking of IL13, peptide or antibody: Fiberless radiative effectors are produced by stirring the PEG linked liposomes with NHS-biotin in saline at room temperature under nitrogen overnight. The biotinylated liposomes are then incubated with avidin linked IL13, and incubated for three hours at room temperature. The final product is rinsed and filtered with phosphate buffer.

Example 12

Synthesis of Fiberless Radiative Effector Containing a Bioluminescent Protein

This example describes the synthesis of fiberless radiative effectors that contain a bioluminescent protein. These fiberless radiative effectors are used for imaging or to provide a light source for excitation of fiberless radiative effectors containing a photodynamic compound. Briefly, these fiberless radiative effectors are fabricated using the procedure outlined in Example 6 above to prepare PEG cloaked sol-gel fiberless radiative effectors, except that 25 μg luciferase for 50 μg Ruthenium dye. Conjugation of the PEG cloaked

fiberless radiative effectors to biotin is completed by following the procedure described in Example 4.

Example 13

Photodynamic Fiberless Radiative Effector Mediated Cell Death

This example demonstrates that fiberless radiative effectors containing a photodynamic compound are toxic to cells. Human SY5Y neuroblastoma cells lipofected with fiberless radiative effectors containing oxygen-sensitive ruthenium red dye were evaluated for indices of cell death by confocal microscopy upon exposure to laser light. Cells were exposed for 30 min to laser light using the 488 nm line of an Ar-Kr laser on a laser scanning confocal microscope by focusing the beam (attenuated to 60% power and further attenuated by 20% using barrier filters) through a 60X 1.4/NA objective. Subsequent to laser exposure cells were loaded for 15 min at 37°C, with the viability indicator dye calcein (AM) and the cell death indicator dye propidium iodide, and imaged by confocal microscopy using a 20X 0.75/NA objective (488EX/525EM for calcein, 568EX/590EM). The dotted line indicates (See Fig. 2) the demarcation between cells exposed to the light path (60 or 40X) or not exposed (20X). Arrowheads indicate dead (propidium iodide) or dying (morphologic appearance of apoptosis) cells. Cells were grown to confluence prior to illumination. The density of cells within the area of illumination was severely reduced. Even though several of the cells in the irradiated area are not yet dead, they displayed many signs of phototoxicity including "balling-up" of cells and retraction of any neurites and phylopodia which may have developed during culture. Analogous results have been obtained with C6 glioma cells using ruthenium nanospheres. Despite the fact that the neuroblastoma and glioma cells in culture have very different susceptibilities (SY5Y cells do not express Bcl-XL in response to oxidative stress), they respond similarly to the photodynamic effects of fiberless radiative effectors.

Example 14

Non-Specific Liposomal Delivery of PEBBLEs

Liposomal delivery is accomplished using commercially available liposomes called ESCORT (composed of a 1:1 mixture of cationic lipids, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoyl phosphatidylethanolamine). The liposomes incorporate into the cell membrane and empty their contents (*e.g.*, PEBBLEs; Probes Encapsulated By Biologically Localized Embedding) into the cytoplasm of the cell. Liposomal delivery has proven to be an effective method for inserting a large amount of PEBBLEs into cells, unlike the gene gun which only injects one or two PEBBLEs into a cell. Additionally, a large number of cells can be injected at once, in contrast to pico-injection, which is useful only on a cell-by-cell basis. ESCORT was used effectively to insert calcium selective PEBBLEs into neuroblastoma cells (Fig. 3; (a) Nomarski illumination; (b) fluorescence image with excitation at 590 nm).

Example 15

In Situ Imaging of Tumors by MRI

In this example, MRI was used to image non-specific monocrystalline iron oxide (MION) particles in tumor cells in rats. In these experiments, rats with intracerebral 9L tumors were injected in the tail vein with MION particles at a dose of 15 mg Fe/kg body weight. T1 and T2-weighted MR imaging was performed over time pre-and 1, 2 and 3 days post-MION administration on a 7-Tesla MR system. A time series of *in vivo* T1-weighted MR images from a single animal harboring a 9L tumor were collected. At 24 hours following MION administration, the contrast in the tumor but not in the contralateral brain tissue was dramatically altered by the presence of MION as evidenced by the shortening of the T1 relaxation time resulting in an increase in the MR T1 signal intensity. At 2 days following MION administration, the change in signal contrast had almost completely returned to that observed in the pre-MION tumor except for the nanoparticles left in the necrotic region of the tumor (right side). Next, MR images were from the same animal, but with T2-weighting. In

this case (T2-weighted imaging), the presence of MION was revealed by the loss of signal intensity and the fact that the tumor turns dark to black in regions where the MION particles have accumulated. T2-weighted images appear a bit more sensitive as the change in signal persists into days 2-3 post MION-administration. Histological examination of sections stained for hematoxylin and DAB amplified Perls' confirmed the dynamic time-course for uptake and elimination of MION in the tumor tissue and the lack of entrance into the normal brain parenchyma. The selectivity of entrance into the tumor tissue is due to the disrupted blood brain barrier within the tumor mass. This data reveals that MR imaging, especially T2-weighted imaging can clearly be used to non-invasively follow the uptake and clearance of nanoparticles within tumor tissue. It is contemplated that targeting prolongs the half-life of nanoparticles in the tumor, thereby enhancing imaging in therapeutic regimens.

Example 16

In Situ Evaluation of Tumor Therapy by MRI

To demonstrate the utility of MRI for non-invasive quantitation of therapeutic efficacy, rats with intracerebral 9L brain tumors were treated with BCNU and used serial MRI to follow tumor growth over time. In summary, these experiments revealed that tumor cell log kills can be quantitated in individual animals. Shown in Figures 4 A, B and C are plots of representative intracranial tumor volumes versus time, each plot taken from a single animal, both pre- and post-treatment for 0.5, 1, and 2 LD10 doses of BCNU. The individual volume measurements are shown along with the pre- (solid line) and post-treatment (dashed line) exponential regrowth curve fits following treatment with (A) 0.5, (B) 1.0, and (C) 2.0 times LD10 BCNU. Pre- and post-BCNU doubling times (td) are also shown, revealing a substantial decrease in growth rate during tumor regrowth.

Tumor growth slowed immediately following BCNU treatment and the time to regrowth increased with increasing BCNU dose. An exceptional finding was that tumors re-grow at a significantly slower rate following treatment. This fact was previously unknown, even after extensive research reports in the literature using the 9L tumor model to study the effects of

BCNU. Log kill values were obtained from these data for each individual animal by extrapolation of the regrowth line back to the time of treatment as denoted by Tx in Figure 4. Using MRI tumor volumetric data, it was found that 9L tumor log kill values were 0.2±0.1 (±S.D., n=5), 1.0±0.3 (n=4) and 1.7±0.6 (n=6), respectively for 0.5, 1 and 2 LD10 BCNU doses which are considerably less (Ross *et al.*, PNAS 95:7012-17 [1998]) than the 1.7, 3.2 and 3.7 log kills derived from traditional measurements (Rosenblum *et al.*, J. Neurosurg. 46:145-54 [1997]). It is not intended that the present invention be limited to particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to make and use the present invention. However, the differences in calculated cell kills are largely attributable to the fact that it was assumed that regrowth and untreated tumor growth kinetics were equivalent. Moreover, the slower tumor re-growth kinetics following treatment reveals that increases in animal survival cannot be attributed solely to the proportion of tumor cell killed. While it is true that more cells are killed at higher doses of BCNU, this does not appear to be the dominant reason for the delay in tumor progression. It appears that the slower growth rate following treatment effects tremendously the time to repopulate the tumor. This data shows the power of the MRI approach for following the effects of therapy on intracranial tumor growth.

Example 17

Detection of Biological Targets with Fiberless Radiative Effector Containing Luciferase

This example describes the utility of fiberless radiative effectors containing the useful radiative beacon, luciferase. Confocal fluorescence images of DNPs containing luciferase encapsulated in sol gel matrices are obtained using the Olympus AX70 fluorescence microscope fitted with a Spectramaster monochromater and spinning disc Confocal attachment. Green or red fluorescence of luciferin are excited at the appropriate wavelength using the liquid fiber-optic light guide of the monochromator. Emitted fluorescence are collected using a 60X-1.4 NA oil-immersion objective lens. Data is acquired and analyzed using the LSR Merlin and Esprit Software on a dedicated Dell 400 MHz Pentium II MMX

computer with 1Gb RAM. Sol-gel fiberless radiative effectors are delivered to cells in culture using liposomal techniques. The specificity of light produced is determined by depleting cells of ATP using p-nitrophenol to uncouple mitochondria.

Example 18

Targeted Destruction of Tumor Cells

This example describes the destruction of tumor cells by fiberless radiative effectors conjugated to tumor cell specific molecular recognition elements. The RGD peptide (CDCRGDCFC; SEQ ID NO:1) and IL13 will be conjugated to fiberless radiative effectors as described.

Confocal Microscopy Analysis of Apoptosis: To evaluate onset of apoptosis, cultured C6 glioma and SY5Y neuroblastoma cells grown on 22 mm glass coverslips are incubated and exposed to DNPs and loaded for 15 min with 1 M calcein acetoxymethyl ester (AM). Following dye-loading, cells are washed once with incubation buffer (Dulbecco's phosphate buffered saline containing $CaCl_2$, 0.1 g/L, glucose, 1 g/L and sodium pyruvate, 0.036 g/L), mounted on the microscope stage in incubation buffer containing 3 M propidium iodide and incubated at 37°C. Sequential confocal images will be obtained for cytosolic (calcein) and nuclear (propidium iodide) staining to evaluate morphologic changes associated with apoptosis. For longer treatment time-points (beyond 1 hr), cells are loaded with dyes as described above and single confocal images obtained for calcein and propidium iodide. Confocal fluorescence images of calcein and propidium iodide are obtained using the Olympus AX70 fluorescence microscope fitted with a Spectramaster monochromater and Confocal attachment. Green fluorescence of calcein and red fluorescence of propidium iodide is excited simultaneously at 488 and 568-nm using the liquid fiber-optic light guide of the monochromator. Emitted fluorescence is monitored using a 60X-1.4 NA oil-immersion objective lens. Data will be acquired and analyzed using the LSR Merlin and Esprit Software on a dedicated Dell 400 MHz Pentium II MMX computer with 1Gb RAM.

DNA Laddering Analysis of Apoptosis: Cells treated as described above are harvested for genomic DNA isolation using the QiaPrep DNA isolation system (Qiagen, Chatsworth, CA). Genomic DNA samples are resolved by electrophoresis on 1.5% agarose gels in Tris-Borate-EDTA (1X TBE) buffer containing ethidium bromide (50 ng/ml) for 1.5 hours at 50 mV. The appearance of discreet 160 kb bands in DNA samples from cells undergoing apoptosis is visualized by UV illumination and polaroid photography.

Example 19

In Situ Monitoring of Fiberless Radiative Effector Therapy

This example describes the *in situ* monitoring in rats of tumor therapy using fiberless radiative effectors.

Cell Culture Conditions: Both SCC VII (mouse squamous cell carcinoma of the head and neck) and 9L (human gliosarcoma) tumor cells are grown as monolayers in 75-cm^2 sterile plastic flasks in modified Eagle's minimum essential medium with 10% fetal calf serum. Cells are cultured in an incubator at 37°C in an atmosphere containing 95% air and 5% CO_2 until confluent. The cells are harvested, counted and concentrated in serum-free media for tumor implantation.

Stable Transfection: Stable 9L and SCCVII cell lines are constructed which stably carry and express the luciferase gene. Animals are implanted with these lines at their respective sites (brain and submental region of the mouth) and imaged twice a week for six weeks. To validate that the bioluminescence results are reproducible measures of tumor burden, MRI data is collected.

Both cell lines are transfected with pgLUC/CMV by the calcium phosphate precipitation method. Exponentially growing cells will be split 1:10 onto 10-cm tissue culture dishes the day before transfection with 30 μg of plasmid. Stable transfectants are selected by growth in 500 μM G418 for approximately two weeks. Luciferase expression from polyclonal transfectants from multiple transfections is analyzed as described below. Luciferase

expression from monoclonal lines isolated from serial dilution is assayed in a 96-well plate using a Perkin Elmer LS50B bioluminometer.

Induction of Intracranial and Head & Neck Tumors: Intracerebral brain tumors are induced in anesthetized male Fischer-344 rats. A small skin incision is made through the scalp and a 1 mm-diameter hole drilled through the cranium using a high speed drill. A suspension of $1x10^5$ tumor cells in 10 µl serum-free culture media is slowly injected into the right frontal lobe at a depth of 2.5 mm from the dural surface. The burr hole is filled with bone wax and the incision closed with suture.

In the experiments described here, C3H mice are utilized as hosts and SCCVII cells as the model squamous cell tumor. Cells are harvested from 150 mm tissue culture dishes by scraping, washing in PBS, and resuspending in media at 1.2×10^8 cells/ml. C3H mice aged 6-10 weeks and weighing 25-30 grams are anesthetized by i.p. injection of 0.5 ml avertin at a concentration of 20 mg/ml. Fifty µl of the cell suspension will be injected into the submental region using a 26 gauge syringe.

Magnetic resonance imaging (MRI): All *in vivo* MR experiments are performed on a Varian Imaging system equipped with a 7.0 Tesla, 18.3 cm horizontal bore magnet (300 MHz proton frequency). Rat brains harboring intracerebral tumors are imaged at approximately 10-30 days following cell implantation in two day intervals. Each MRI session includes: (a) Single slice gradient-recalled-echo scanning with 1 mm "saturation cross-hairs" imprinted on the axial and coronal images to facilitate rapid and reproducible positioning of the coil and rigid animal support rig (ear posts and bite bar). T2-weighted images through the rat brain are produced using the following parameters: TR/TE = 3500/60, FOV= 30 x 30 mm using a 128 x 128 matrix, slice thickness = 0.5 mm, number of slices and slice separation = 0.8 mm.

Bioluminescent Imaging of Tumors: A Hamamatsu intensified CCD camera (Model C2400-32) is used for imaging of tumor bearing animals. Tumor cells constitutively expressing luciferase are grown in the brain or neck of animals. At the time of imaging, animals are injected with luciferin i.p. at a dose of 150 mg/kg as described previously (Contag *et al., supra*). Animals are anesthetized and placed in the imaging chamber and data will be

acquired for 5-10 minutes depending upon tumor burden. Data is processed off-line using software provided by Dr. Chris Contag, Stanford University.

As is evident from the above, the present invention provides a viable alternative strategy and reagents for photodynamic therapy. The fiberless radiative effectors of the present invention can be administered in low doses, so that collateral damage of adjacent tissues or cells is low, and so that cutaneous photosensitivity should be reduced.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology, genetics, materials sciences, polymer sciences or related fields are intended to be within the scope of the following claims.

TARGETED FIBERLESS RADIATIVE EFFECTORS

What is claimed is:

1. A composition comprising a molecular recognition element attached to a fiberless radiative effector, said effector comprising a toxic agent.

2. The composition of Claim 1, wherein said molecular recognition comprises an antigen binding protein.

3. The composition of Claim 1, wherein said molecular recognition element comprises a nucleic acid.

4. The composition of Claim 1, wherein said molecular recognition element comprises a peptide.

5. The composition of Claim 1, wherein said molecular recognition element comprises biotin.

6. The composition of Claim 1, wherein said fiberless radiative effector further comprises a polymer.

7. The composition of Claim 6, wherein said polymer is selected from poly(vinyl chloride), poly(vinyl chloride) carboxylated and poly(vinyl chloride-co-vinyl acetate co-vinyl) alcohols.

8. The composition of Claim 1, wherein said fiberless radiative effector further comprises a plasticizer.

9. The composition of Claim 1, wherein said toxic agent comprises a photosensitizer.

10. The composition of Claim 1, wherein said toxic agent comprises a radioactive element.

11. The composition of Claim 1, further comprising a cloaking material.

12. The composition of Claim 11, wherein said cloaking material is selected from poly(ethylene glycol) and a liposome.

13. The composition of Claim 11, wherein said cloaking material is a liposome, said liposome containing a plurality of said fiberless radiative effectors.

14. The composition of Claim 12, wherein said molecular recognition element is attached to said liposome.

15. A method comprising:

1) providing

i) a biological target, and

ii) a first fiberless radiative effector comprising a molecular recognition element and a toxic agent; and

2) combining said target and said fiberless radiative effector cell under conditions such that said molecular recognition element binds to said target to form a first effector-target complex.

16. The method of Claim 15, wherein said target is selected from a cellular receptor, a polypeptide, an antigen, a nucleic acid, and biotin.

17. The method of Claim 15 wherein said toxic agent comprises a photosensitizer.

18. The method of Claim 17, wherein said photosensitizer dye is selected from Photofrin, Ru-diphenyl-phenanthroline, Tris(1-10-phenanthroline)ruthenium(II) chloride), tin ethyl etiopurpurin, protoporphyrin IX, chloroaluminum phthalocyanine, tetra(M-hydroxyphenyl)chlorin

19. The method of Claim 18, further comprising exposing said first effector-target complex to a light source under conditions such that singlet oxygen is produced.

20. The method of Claim 15, further comprising the steps of:

3) providing a second fiberless radiative effector comprising a second molecular recognition element, and

4) combining said first effector-target complex with said second fiberless radiative effector under conditions such that said second molecular recognition element binds to form a target-second effector-target complex.

21. The method of Claim 20, wherein said toxic agent comprises a photosensitizer.

22. The method of Claim 21, wherein said second fiberless radiative effector comprises a radioactive element.

23. A method comprising:

1) providing

i) a biological target, and

ii) a first fiberless radiative effector comprising a molecular recognition element and a marker; and

2) combining said target and said fiberless radiative effector cell so that said molecular recognition element binds to said target form a target-first fiberless radiative effector complex.

24. The method of Claim 23, wherein said marker is a protein that catalyzes a luminescent reaction.

25. The method of Claim 23 further comprising the step of

3) exposing said target-first fiberless radiative effector complex to a substrate so that luminescence is produced.

26. The method of Claim 23, wherein said marker is a magnetic material.

27. The method of Claim 26 further comprising the step of

3) detecting said magnetic material by magnetic resonance imaging.

28. A composition comprising a molecular recognition element attached to a liposome, said liposome containing a plurality of fiberless radiative effectors comprising a toxic agent.

29. The composition of Claim 28, wherein said molecular recognition comprises an antigen binding protein.

30. The composition of Claim 31, wherein said molecular recognition element comprises a nucleic acid.

31. The composition of Claim 28, wherein said molecular recognition element comprises a peptide.

32. The composition of Claim 28, wherein said molecular recognition element comprises biotin.

33. The composition of Claim 28, wherein said liposome further comprises poly(ethylene glycol).

34. The composition of Claim 28, wherein said fiberless radiative effector further comprises a polymer.

35. The composition of Claim 34, wherein said polymer is selected from poly(vinyl chloride), poly(vinyl chloride) carboxylated and poly(vinyl chloride-co-vinyl acetate co-vinyl) alcohols.

36. The composition of Claim 28, wherein said fiberless radiative effector further comprises a plasticizer.

37. The composition of Claim 28, wherein said toxic agent comprises a photosensitizer.

38. The composition of Claim 28, wherein said toxic agent comprises a radioactive element.

Figure 1

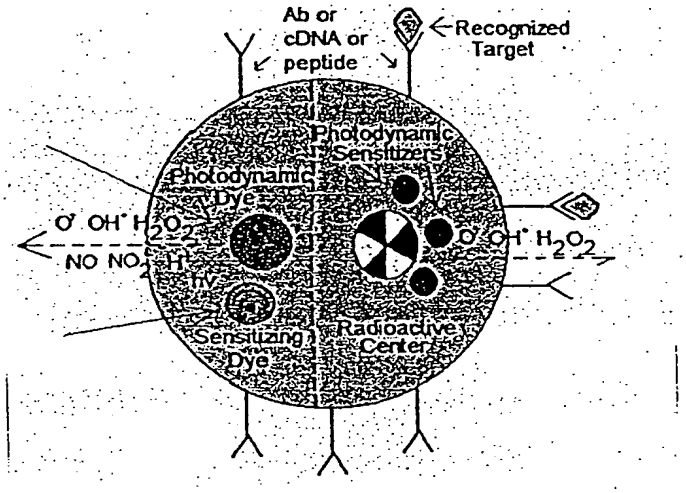


Figure 2

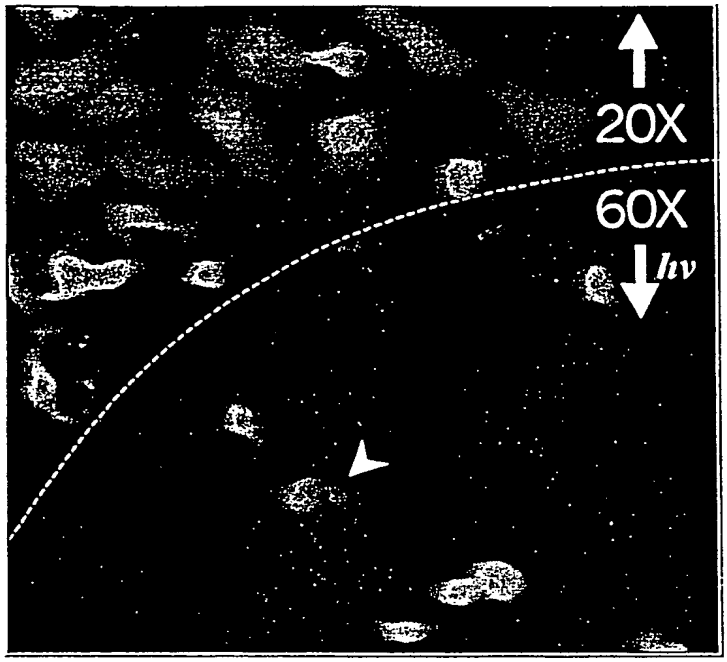
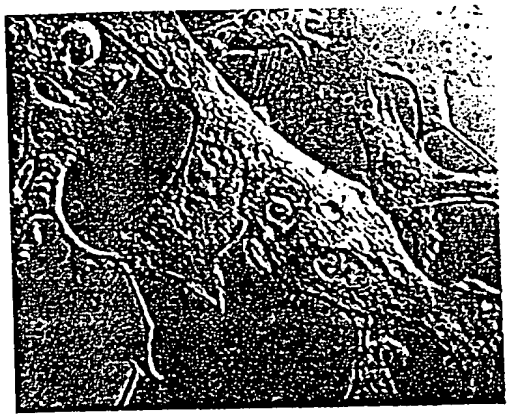
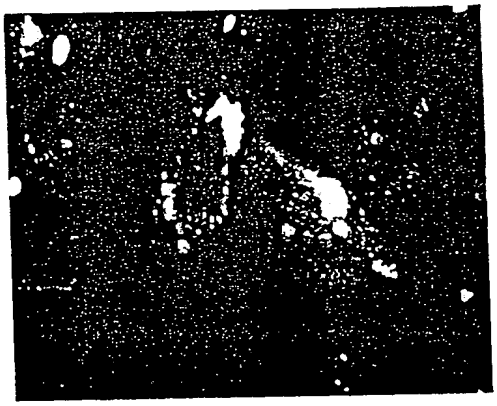


Figure 3

a



b



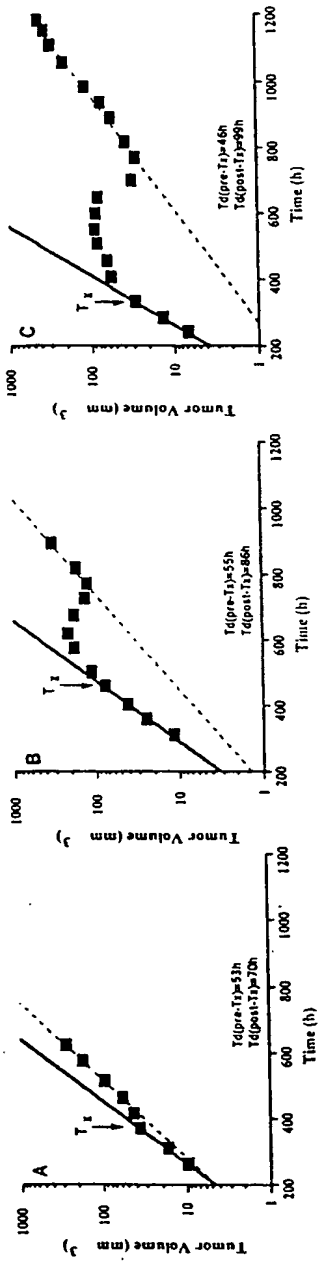


Figure 4

Figure 5

A. Human IL13 cDNA

```
   1 aagccaccca gcctatgcat ccgctcctca atcctctcct gttggcactg ggcctcatgg
  61 cgcttttgtt gaccacggtc attgctctca cttgccttgg cggctttgcc tccccaggcc
 121 ctgtgcctcc ctctacagcc ctcagggagc tcattgagga gctggtcaac atcacccaga
 181 accagaaggc tccgctctgc aatggcagca tggtatggag catcaacctg acagctggca
 241 tgtactgtgc agccctggaa tccctgatca acgtgtcagg ctgcagtgcc atcgagaaga
 301 cccagaggat gctgagcgga ttctgcccgc acaaggtctc agctgggcag ttttccagct
 361 tgcatgtccg agacaccaaa atcgaggtgg cccagtttgt aaaggacctg ctcttacatt
 421 taaagaaact ttttcgcgag ggacggttca actgaaactt cgaaagcatc attatttgca
 481 gagacaggac ctgactattg aagttgcaga ttcatttttc tttctgatgt caaaaatgtc
 541 ttgggtaggc gggaaggagg gttagggagg ggtaaaattc cttagcttag acctcagcct
 601 gtgctgcccg tcttcagcct agccgacctc agccttcccc ttgcccaggg ctcagcctgg
 661 tgggcctcct ctgtccaggg ccctgagctc ggtggaccca gggatgacat gtccctacac
 721 ccctcccctg ccctagagca cactgtagca ttacagtggg tgccccctt gccagacatg
 781 tggtgggaca gggacccact tcacacacag gcaactgagg cagacagcag ctcaggcaca
 841 cttcttcttg gtcttattta ttattgtgtg ttatttaaat gagtgtgttt gtcaccgttg
 901 gggattgggg aagactgtgg ctgctggcac ttggagccaa gggttcagag actcagggcc
 961 ccagcactaa agcagtggac cccaggagtc cctggtaata agtactgtgt acagaattct
1021 gctacctcac tggggtcctg gggcctcgga gcctcatccg aggcagggtc aggagagggg
1081 cagaacagcc gctcctgtct gccagccagc agccagctct cagccaacga gtaatttatt
1141 gttttctcctc gtatttaaat attaaatatg ttagcaaaga gttaatatat agaagggtac
1201 cttgaacact gggggagggg acattgaaca agttgtttca ttgactatca aactgaagcc
1261 agaaataaag ttggtgacag ata
```

B. Human IL13 protein sequence- wildtype mature peptide

```
  1 spgpvppsta lrelieelvn itqnqkaplc ngsmvwsinl tagmycaale
 51 slinvsgcsa iektqrmlsg fcphkvsagq fsslhvrdtk ievaqfvkdl
101 llhlkklfre grfn
```

C. hIL13.E13K protein sequence

```
  1 spgpvppsta lrklieelvn itqnqkaplc ngsmvwsinl tagmycaale
 51 slinvsgcsa iektqrmlsg fcphkvsagq fsslhvrdtk ievaqfvkdl
101 llhlkklfre grfn
```

D. hIL13.E13Y protein sequence

```
  1 spgpvppsta lrylieelvn itqnqkaplc ngsmvwsinl tagmycaale
 51 slinvsgcsa iektqrmlsg fcphkvsagq fsslhvrdtk ievaqfvkdl
101 llhlkklfre grfn
```

SEQUENCE LISTING

<110> Philbert, Martin A.
Tjalkens, Ronald
Aylott, Jonathan W.
Clark, Heather A.
Monson, Eric E.
Kopelman, Raoul

<120> Targeted Fiberless Radiative Effectors

<130> UM-03686

<140> 09/366,314
<141> 1999-08-02

<160> 9

<170> PatentIn Ver. 2.0

<210> 1
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 1
Cys Asp Cys Arg Gly Asp Cys Phe Cys
1 5

<210> 2
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 2
Cys Asn Gly Arg Cys Val Ser Gly Cys Ala Gly Arg Cys
1 5 10

<210> 3
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 3
Gly Ser Leu
1

<210> 4
<211> 114
<212> PRT
<213> Homo sapiens

<400> 4
Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu
1 5 10 15

Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
20 25 30

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
35 40 45

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
50 55 60

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65 70 75 80

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
85 90 95

Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
100 105 110

Phe Asn

<210> 5
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 5
Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Lys Leu Ile Glu
1 5 10 15

Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
20 25 30

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
35 40 45

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
50 55 60

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65 70 75 80

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
85 90 95

Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
100 105 110

Phe Asn

<210> 6
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 6
Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Tyr Leu Ile Glu
1 5 10 15

Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
20 25 30

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
35 40 45

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
50 55 60

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65 70 75 80

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
85 90 95

Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
100 105 110

Phe Asn

<210> 7
<211> 1283
<212> DNA
<213> Homo sapiens

<400> 7
aagccaccca gcctatgcat ccgctcctca atcctctcct gttggcactg ggcctcatgg 60
cgcttttgtt gaccacggtc attgctctca cttgccttgg cggctttgcc tccccaggcc 120
ctgtgcctcc ctctacagcc ctcagggagc tcattgagga gctggtcaac atcacccaga 180
accagaaggc tccgctctgc aatggcagca tggtatggag catcaacctg acagctggca 240
tgtactgtgc agccctggaa tccctgatca acgtgtcagg ctgcagtgcc atcgagaaga 300
cccagaggat gctgagcgga ttctgcccgc acaaggtctc agctgggcag ttttccagct 360
tgcatgtccg agacaccaaa atcgaggtgg cccagtttgt aaaggacctg ctcttacatt 420
taaagaaact ttttcgcgag ggacggttca actgaaactt cgaaagcatc attatttgca 480
gagacaggac ctgactattg aagttgcaga ttcatttttc tttctgatgt caaaaatgtc 540
ttgggtaggc gggaaggagg gttagggagg ggtaaaattc cttagcttag acctcagcct 600
gtgctgcccg tcttcagcct agccgacctc agccttcccc ttgcccaggg ctcagcctgg 660
tgggcctcct ctgtccaggg ccctgagctc ggtggaccca gggatgacat gtccctacac 720
ccctcccctg ccctagagca cactgtagca ttacagtggg tgccccctt gccagacatg 780
tggtgggaca gggacccact tcacacacag gcaactgagg cagacagcag ctcaggcaca 840
cttcttcttg gtcttattta ttattgtgtg ttatttaaat gagtgtgttt gtcaccgttg 900
gggattgggg aagactgtgg ctgctggcac ttggagccaa gggttcagag actcagggcc 960
ccagcactaa agcagtggac cccaggagtc cctggtaata agtactgtgt acagaattct 1020
gctacctcac tggggtcctg gggcctcgga gcctcatccg aggcagggtc aggagagggg 1080
cagaacagcc gctcctgtct gccagccagc agccagctct cagccaacga gtaatttatt 1140
gtttttcctc gtatttaaat attaaatatg ttagcaaaga gttaatatat agaagggtac 1200
cttgaacact gggggagggg acattgaaca agttgtttca ttgactatca aactgaagcc 1260
agaaataaag ttggtgacag ata 1283

<210> 8
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 8
Met Leu Ser Leu Arg Gln Ser Ile Arg Phe Phe Lys Pro Ala Thr Arg
1 5 10 15

Thr Leu

<210> 9
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 9
Pro Pro Lys Lys Lys Arg Lys Val
1 5

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/08660 A3

(51) International Patent Classification[7]: A61K 51/12, 41/00, 49/00

(21) International Application Number: PCT/US00/20292

(22) International Filing Date: 26 July 2000 (26.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/366,314 2 August 1999 (02.08.1999) US

(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 3003 South State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PHILBERT, Martin, A.** [GB/GB]; 48311 Hill Top Drive East, Plymouth, MI 48170 (US). **TJALKENS, Ronald** [US/US]; 4259 Dexter Road, Ann Arbor, MI 48103 (US). **AYLOTT, Jonathan, W.** [US/US]; 2170 Cram Place #13, Ann Arbor, MI 48105 (US). **CLARK, Heather, A.** [US/US]; 168 Dove Lane, Middletown, CT 06457 (US). **MONSON, Eric, E.** [US/US]; 2025 Huron Parkway #211, Ann Arbor, MI 48104 (US). **KOPELMAN, Raoul** [US/US]; 1065 Heathcrway Street, Ann Arbor, MI 48104 (US).

(74) Agents: **CARROLL, Peter, G.** et al.; Medlen & Carroll, LLP, Suite 2200, 220 Montgomery Street, San Francisco, CA 94104 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
22 November 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/08660 A3

(54) Title: TARGETED FIBERLESS RADIATIVE EFFECTORS

(57) Abstract: The present invention is related to cell or pathogen destruction via fiberless radiative effectors that encapsulate a free radical generator. Fiberless radiative effectors include a polymer matrix, a photodynamic or radiodynamic dye which produces free radicals upon stimulation, cloaking material, and at least one molecular recognition element for targeting to a biological target. Other fiberless radiative effectors may be used for imaging biological targets, and include a compound which is detectable by an imaging system, such as luciferase or iron.

INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/US 00/20292

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K51/12 A61K41/00 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 02651 A (UNIV MICHIGAN) 21 January 1999 (1999-01-21) page 9, line 8 - line 15 page 15, line 7 - line 10; claims 1,6,16; example 5 ---	1-38
Y	WO 97 46262 A (PHARMACYCLICS INC ;MAGDA DARREN (US); MODY TARAK D (US); UNIV TEXA) 11 December 1997 (1997-12-11)	1-38
X	claims ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2001	12/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/20292

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLARK H A ET AL: "OPTOCHEMICAL NANOSENSORS AND SUBCELLAR APPLICATIONS IN LIVING CELLS" MIKROCHIMICA ACTA,SPRINGER VERLAG, VIENNA,AT, vol. 131, no. 1/02, 1999, pages 121-128, XP000992957 ISSN: 0026-3672	23-27
Y	abstract; figure 1 page 124, column 2, paragraph 3 ---	1-38
A	US 5 599 923 A (HEMMI GREGORY W ET AL) 4 February 1997 (1997-02-04) column 23, line 15 - line 31; claims column 11, line 21 - line 27 column 4, line 28 - line 53 ---	1-38
A	WO 96 23524 A (COCKBAIN JULIAN R M ;FUGLAAS BJORN (NO); KLAVENESS JO (NO); RONGVE) 8 August 1996 (1996-08-08) page 14, paragraph 4 -page 15, paragraph 1; claims; examples 5,6,8 page 19, paragraph 4 -page 21, paragraph 1 page 24, paragraph 2 -page 25, paragraph 2 page 27, paragraph 2 - paragraph 3 ---	23-27
E	WO 00 43045 A (PHOTOGEN INC) 27 July 2000 (2000-07-27) claims 1,5,7,38,39,45,57,60 -----	1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 15-22 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claim(s) 23-27 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Present claims 1-38 relate to an extremely large number of possible compounds or products. In fact, the claims contain so many options or variables that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the examples .

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/20292

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902651 A	21-01-1999	US 6143558 A	07-11-2000
		AU 8282398 A	08-02-1999
WO 9746262 A	11-12-1997	AU 727138 B	07-12-2000
		AU 3226497 A	05-01-1998
		BR 9710685 A	11-01-2000
		CA 2257225 A	11-12-1997
		CN 1225591 A	11-08-1999
		EP 0954336 A	10-11-1999
		JP 2000512279 T	19-09-2000
		NO 985645 A	03-02-1999
US 5599923 A	04-02-1997	US 5569759 A	29-10-1996
		US 5457183 A	10-10-1995
		US 5252720 A	12-10-1993
		US 5162509 A	10-11-1992
		US 4935498 A	19-06-1990
		AU 688008 B	05-03-1998
		AU 1921795 A	29-08-1995
		CA 2182960 A	17-08-1995
		EP 0745085 A	04-12-1996
		JP 10500659 T	20-01-1998
		NO 963396 A	14-10-1996
		NZ 282185 A	25-03-1998
		WO 9521845 A	17-08-1995
		US 5599928 A	04-02-1997
		US 5994535 A	30-11-1999
		US 5504205 A	02-04-1996
		US 5733903 A	31-03-1998
		US 5801229 A	01-09-1998
		US 6069140 A	30-05-2000
		AU 683316 B	06-11-1997
		AU 8075694 A	04-05-1995
		CA 2173319 A	20-04-1995
		EP 0724457 A	07-08-1996
		JP 9508616 T	02-09-1997
		NO 961436 A	11-06-1996
		SG 48392 A	17-04-1998
		WO 9510307 A	20-04-1995
		US 6072038 A	06-06-2000
		US 5622946 A	22-04-1997
		US 5583220 A	10-12-1996
		US 5580543 A	03-12-1996
		US 5587371 A	24-12-1996
		US 5632970 A	27-05-1997
		AU 3436793 A	03-08-1993
		CA 2127530 A	22-07-1993
		EP 0623134 A	09-11-1994
		FI 943445 A	20-07-1994
		JP 7503009 T	30-03-1995
		NO 942719 A	19-09-1994
		NZ 246795 A	27-02-1996
		WO 9314093 A	22-07-1993
		US 5432171 A	11-07-1995
		US 5475104 A	12-12-1995
		US 5439570 A	08-08-1995
		US 5451576 A	19-09-1995
		US 5559207 A	24-09-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/20292

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5599923 A		US 5567687 A	22-10-1996
		US 5607924 A	04-03-1997
		US 5595726 A	21-01-1997
		US 5763172 A	09-06-1998
WO 9623524 A	08-08-1996	AU 4545896 A	21-08-1996
		BR 9607012 A	28-10-1997
		CA 2212257 A	08-08-1996
		EP 0808175 A	26-11-1997
		FI 973208 A	01-10-1997
		HU 9900610 A	28-07-1999
		JP 10513175 T	15-12-1998
		NO 973542 A	01-10-1997
WO 0043045 A	27-07-2000	AU 2972500 A	07-08-2000